1982

cancer treatment reports

RIECKER MEMORIAL LIBRARY

December 1982
Volume 66
Number 12
Pages 2005-2149

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Vincent T. DeVita, Jr., ***Director***
Division of Cancer Treatment, Bruce A. Chabner, ***Director***
Scientific Information Branch, Susan Molloy Hubbard, ***Chief***

Editorial Board

John S. Macdonald, ***Editor-In-Chief***

Associate Editors

Martin D. Abeloff
Joseph Aisner
Lewis Aronow
Richard L. Cysyk
Ross C. Donehower
William D. DeWys
Daniel C. Ihde
Allen S. Lichter
Robert W. Makuch
David P. Pistenmaa
Philip A. Pizzo
Charles A. Schiffer
Mary K. Wolpert

Advisory Board

Gianni Bonadonna
George P. Canellos
Richard H. Creech
Harvey M. Golomb
Robert L. Goodman
Stephen B. Howell
Randall K. Johnson
Irwin H. Krakoff
Brigid G. Leventhal
Robert B. Livingston
Albert F. LoBuglio
William L. McGuire
Franco M. Muggia
Carol S. Portlock
Sydney E. Salmon

Editorial Staff

Jean Griffin Baum, ***Managing Editor***
Dee Kittner LeRoy, ***Assistant Managing Editor***

Editors

Elaine Price Beck
Anne McCarthy
Marian Sharp McGrath

Editorial Assistants

Elaine Brown
Susan Lee Gordon
Jana Johnston
Barbara Lineberry

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave, Rm 8C08, Landow Bldg, Bethesda, MD 20814 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears in the second page of every issue.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

INSTRUCTIONS TO AUTHORS

Title Page
The complete title of the manuscript and the full name, affiliation, and address of each author should be included on the title page. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city and name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names or The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. A second table will be permitted only when requested by the editorial board. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints
Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

Contents

Cancer Treatment Reports
Volume 66, Number 12
December 1982

Clinical and Phase I–II Studies

2005 Random Prospective Study of Cyclophosphamide, Doxorubicin, and Methotrexate (CAM) Combination Chemotherapy Versus Single-Agent Sequential Chemotherapy in Non-Small Cell Lung Cancer. **L. H. Einhorn, S. D. Williams, E. E. Stevens, W. H. Bond, and L. Chenoweth.** Indiana University Medical Center, Indianapolis

2013 Cisplatin Therapy in Recurrent Childhood Brain Tumors. **A. B. Khan, B. J. D'Souza, M. D. Wharam, L. A. A. Champion, L. F. Sinks, S. Y. Woo, D. C. McCullough, and B. G. Leventhal.** The Johns Hopkins University, Baltimore, Maryland

2021 Comparative Trial of Two Teniposide-Based Combination Chemotherapy Regimens for the Treatment of Advanced Malignant Lymphomas. **M. J. O'Connell, J. Anderson, J. M. Merrill, J. M. Bennett, J. H. Glick, C. W. Berard, R. S. Neiman, and H. S. Brodovsky.** Eastern Cooperative Oncology Group

2027 Phase I Trial of *Staphylococcus aureus* Cowan I Immunoperfusion. **G. Messerschmidt, C. Bowles, D. Dean, M. Parker, R. Lester, R. Dowling, T. Holohan, L. Osborne, B. F. Schaff, K. McCormack, R. Corbitt, T. Phillips, E. Glatstein, and A. Deisseroth.** National Cancer Institute, Bethesda, Maryland

2033 Doxorubicin-DNA Complex: A Phase I Clinical Trial. **S. W. Hall, R. S. Benjamin, M. A. Burgess, G. P. Bodey, M. A. Luna, and E. J Freireich.** University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

2039 Phase I Study of SOAz. **S. Nasca, D. Jezekova, P. Coninx, E. Garbe, Y. Carpentier, and A. Cattan.** Institut Jean Godinot, Reims, France

Clinical and Preclinical Pharmacology

2043 Medroxyprogesterone Acetate (MAP) Relative Bioavailability After Single High-Dose Administration in Cancer Patients. **F. Pannuti, C. M. Camaggi, E. Strocchi, M. Giovannini, A. R. Di Marco, and B. Costanti.** Ospedale M. Malpighi, Bologna, Italy

2051 Comparison of Thymidine and Folinic Acid Protection From Methotrexate Toxicity in Human Lymphoid Cell Lines. **G. P. Browman.** Ontario Cancer Foundation, Hamilton, Canada

2061 Activity of 4'-Epi-doxorubicin on Normal Hematopoietic Precursor Cells in Mice. **A. Sobrero, C. Muzzulini, F. D'Amore, G. Bogliolo, G. Massa, R. Ghio, and I. Pannacciulli.** Istituto Scientifico di Medicina Interna, University of Genoa, Italy

2071 Cytotoxic and Cell Kinetic Effects of 3,4,5-Trihydroxybenzohydroxamic Acid (VF 122) in Hepatoma 3924A Cells. **J. Ban, E. Olah, and G. Weber.** Indiana University School of Medicine, Indianapolis

BRIEF REPORTS

2081 Prednimustine in Combination With Methotrexate and 5-FU (PMF): A Pilot Study. **E. Boesen and H. T. Mouridsen.** Finsen Institute, Copenhagen, Denmark

2085 Cellular and Plasma Kinetics of Daunorubicin Given by Two Methods of Administration in a Patient With Acute Leukemia. **M. W. DeGregorio, C. J. Carrera, J. C. Klock, C. H. Pegelow, and J. R. Wilbur.** Childen's Cancer Research Institute, Pacific Medical Center, and the Cancer Research Institute, University of California, San Francisco

2089 Phase II Study of an Aziridinylbenzoquinone (AZQ) in Disseminated Malignant Melanoma. **E. T. Creagan, A. J. Schutt, D. L. Ahmann, and S. J. Green.** Mayo Clinic, Rochester, Minnesota

2091 Phase II Trial of PALA in Hypernephroma and Urinary Bladder Cancer. **R. B. Natale, A. Yagoda, D. P. Kelsen, R. J. Gralla, and R. C. Watson.** Memorial Sloan-Kettering Cancer Center, New York, New York

CLINICAL TRIALS SUMMARIES

2093 Combination Chemotherapy in Patients With Advanced Colorectal Carcinoma Previously Treated With 5-FU. **H. E. Windschitl, J. E. Krook, L. K. Everson, S. A. Cullinan, and M. Scott.** North Central Cancer Treatment Group

2095 Phase II Study of 5-FU, Doxorubicin, and Mitomycin (FAM) and Chlorozotocin in Advanced Measurable Pancreatic Cancer. **F. P. Smith, V. K. Rustgi, G. Schertz, P. V. Woolley, and P. S. Schein.** Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Washington, DC

2097 Phase II Trial of Aziridinylbenzoquinone (AZQ) in Head and Neck Cancer. **A. A. Forastiere, S. M. Crain, K. Callahan, D. Van Echo, D. Mattox, M. Thant, D. D. Von Hoff, and P. H. Wiernik.** University of Maryland Hospital, Baltimore

2099 Methyl-GAG in Advanced Colon Cancer: A Phase II Trial of the Southwest Oncology Group. **W. A. Knight, III, D. M. Loesch, L. P. Leichman, C. Fabian, and R. M. O'Bryan.** Southwest Oncology Group

2101 Tabular Summary of 1982 Clinical Trials Summaries

LETTERS

2105 Aziridinylbenzoquinone (AZQ) Treatment of Central Nervous System Leukemia. **B. A. Kamen, J. S. Holcenberg, and S. E. Siegel.** Milwaukee Childrens Hospital and Medical College of Wisconsin

2106 Apparent Cyclophosphamide Hypersensitivity: Urticaria Associated With Cyclophosphamide Without Prior Sensitization. **V. Anku.** St. John & Westshore Hospital, Westlake, Ohio

2107 High-Dose Metoclopramide in Cancer Chemotherapy-Induced Nausea and Vomiting. **N. B. Bui, G. Marit, and B. Hoerni.** Fondation Bergonié, Bordeaux, France

2108 High-Dose Metoclopramide: Control of Dacarbazine-Induced Emesis in a Preliminary Trial. **L. B. Tyson, R. A. Clark, and R. J. Gralla.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, New York

2109 Subject Index 1982

2129 Author Index 1982

2141 Reviewers 1982

2147 ANNOUNCEMENTS

RIECKER MEMORIAL LIBRARY
ST. JOSEPH MERCY HOSPITAL
ANN ARBOR, MI 48106

Random Prospective Study of Cyclophosphamide, Doxorubicin, and Methotrexate (CAM) Combination Chemotherapy Versus Single-Agent Sequential Chemotherapy in Non-Small Cell Lung Cancer[1]

Lawrence H. Einhorn,[2,3,*] Stephen D. Williams,[3] Eugene E. Stevens,[3] William H. Bond, and Linda Chenoweth[3]

A group of 104 patients with unresectable non-small cell lung cancer were randomized to receive combination chemotherapy with cyclophosphamide, doxorubicin, and methotrexate (CAM) or single-agent sequential chemotherapy with the same three agents. CAM combination chemotherapy produced a 22% objective response rate, including two complete remissions, compared to a 9% response rate, including one complete remission, produced by single-agent therapy ($P = 0.16$). The median survival time was 32 weeks (range, 3–116) for CAM, compared to 25 weeks (range, 4–179+) for sequential single agents ($P = 0.24$). Overall survival was 31% (1-year), 16% (1½-year), and 5% (2-year), with no difference between the study arms. Although there was no statistically significant survival advantage for the CAM arm, both arms had survival superior to that in historical controls, presumably because of better patient selection. This study indicates that cyclophosphamide, doxorubicin, and methotrexate are, at best, marginally active as single agents, and new drugs with more efficacy are needed before combination chemotherapy can be expected to result in any meaningful prolongation of survival in non-small cell lung cancer. [Cancer Treat Rep 66:2005–2011, 1982]

Lung cancer is a major national health problem and is the most frequent cause of cancer death in the United States. Some 120,000 new cases are expected in 1982, and approximately 90% of these patients will die from their disease.

Although there have been advances during the past 10 years with chemotherapy in small cell lung cancer, progress in non-small cell lung cancer (NSCLC) has been less obvious. A variety of combination chemotherapy regimens have resulted in objective response rates of 25%–50%; however, there is no conclusive evidence that survival is prolonged with combination chemotherapy compared to single-agent chemotherapy or supportive care alone. In 1978, the most common components of these published combination regimens were cyclophosphamide, doxorubicin, and methotrexate (1–3). The purpose of this study was to determine in a stratified, random prospective trial whether there was any advantage in objective response rate or survival for combination chemotherapy with cyclophosphamide, doxorubicin, and methotrexate (CAM) versus single-agent sequential chemotherapy, ie, single-agent cyclophosphamide until disease progression, then doxorubicin, and finally methotrexate.

MATERIALS AND METHODS

From July 1978 to April 1981, 104 patients with histologically confirmed NSCLC were entered in a random prospective study; the schema and dosages for their chemotherapy are given in figure 1. Eligibility requirements were a Karnofsky performance status (KPS) ≥ 50 and no prior chemotherapy with any of the study agents. Patients were stratified according to KPS, limited versus extensive disease, and prior radiotherapy and/or chemotherapy. Limited disease was defined as

[1]Received May 3, 1982; revised Sept 7, 1982; accepted Sept 21, 1982.

[2]Dr. Einhorn is an American Cancer Society Professor of Clinical Oncology.

[3]Department of Medicine, Indiana University Medical Center, Indianapolis.

**Reprint requests to:* Lawrence H. Einhorn, MD, Department of Medicine, Indiana University Medical Center, 926 W Michigan St, University Hospital, Rm 582-N, Indianapolis, IN 46223.

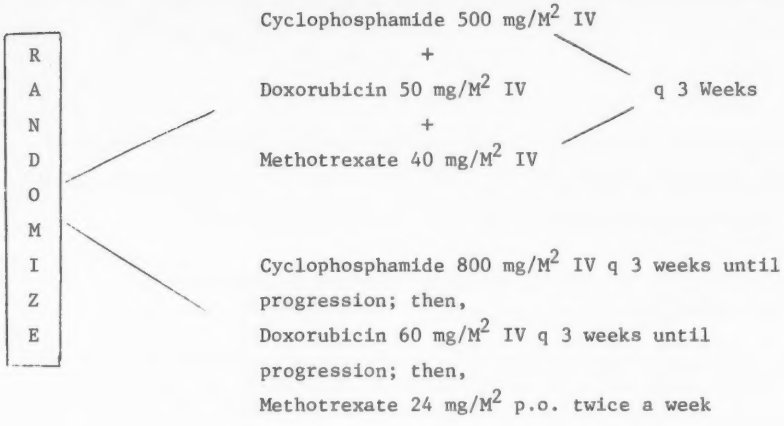
RANDOMIZE
Cyclophosphamide 500 mg/M2 IV
+
Doxorubicin 50 mg/M2 IV
+
Methotrexate 40 mg/M2 IV
q 3 Weeks
Cyclophosphamide 800 mg/M2 IV q 3 weeks until progression; then,
Doxorubicin 60 mg/M2 IV q 3 weeks until progression; then,
Methotrexate 24 mg/M2 p.o. twice a week

FIGURE 1.—Schema and dosages for random prospective study of CAM vs single-agent sequential chemotherapy with the same 3 agents.

disease confined to the hemithorax with or without ipsilateral cervical nodes.

CAM chemotherapy was continued until the total doxorubicin dose was 450 mg/m^2. Thereafter, chemotherapy was continued with cyclophosphamide plus methotrexate for a total of 1 year. Patients randomized to single-agent therapy received cyclophosphamide. After disease progression, the patient was switched to doxorubicin, and after further progression, to methotrexate. All therapy was done on an outpatient basis.

Patient characteristics are shown in table 1. The two groups were comparable, except for a slightly higher unfavorable KPS in the single-agent group and a higher percent of squamous cell lung cancer, but lower percent of large cell lung cancer, in the CAM group. This study was performed at our Veterans Administration Hospital (44% of the patient population) and county hospital (22%) as well as at our university hospital, and the percent of patients treated at each hospital was similar in both arms.

Pretreatment evaluation consisted of medical history; physical examination; cbc; SMA-12; posteroanterior and lateral chest X-ray; and radionuclide scanning of liver, bone, and brain. Tests of measurable disease parameters were repeated every 3 weeks. A complete response (CR) was defined as the disappearance of all clinical evidence of disease, and a partial response (PR) was defined as a 50% decrease in the sum of the products of the perpendicular diameters of measurable disease for at least 1 month or an estimated $\geq$ 75% decrease of poorly defined lesions in the absence of progressive disease elsewhere. Progressive disease was defined as the development of new lesions or a > 25% increase in measurable disease, and stable disease was defined as no remission or progression for a minimum of 3 months. Response duration was calculated from the time of remission to the time of disease progression, and survival was calculated from initiation of CAM or cyclophosphamide therapy to death.

Survival curves were drawn according to Kaplan and Meier (4) and were compared by Gehan's modified Wilcoxon test (5). Differences in proportions between the two groups were tested by the chi-square test (6).

TABLE 1.—Patient characteristics

Characteristic	Sequential single agents (53 patients)	CAM (51 patients)
Age in yrs		
Median	58	58
Range	31–81	42–73
% of patients with—		
KPS		
80–100	38	50
50–70	62	50
Histology		
Adenocarcinoma	36	26
Squamous cell carcinoma	42	69
Large cell carcinoma	22	5
Disease		
Limited	38	40
Extensive	62	60
Prior therapy		
Radiotherapy	13	12
Chemotherapy	8	10

TABLE 2.—Myelosuppression

	CAM	Single-agent therapy
Granulocyte count nadir (cells/mm^3)	1600	1700
< 1000 granulocytes	18%	16%
Platelet count nadir (cells/mm^3)	110,000	140,000

RESULTS

Fifty-one patients were randomized to CAM. Alopecia occurred routinely, and nausea and vomiting was common but not severe. Several patients had dose-limiting stomatitis requiring reduction of their methotrexate dosage. Myelosuppression was generally mild; however, four patients required hospitalization for granulocytopenic fever and presumed infection. There was one episode of fatal doxorubicin-induced congestive heart failure and one episode of hemorrhagic cystitis necessitating termination of cyclophosphamide. Toxic effects of sequential single-agent therapy were generally well-tolerated. Although there was equitoxic myelosuppression from therapy with CAM or single-agent cyclophosphamide, there was more significant alopecia and nausea and vomiting with the combination regimen. The myelosuppression data are shown in table 2.

The therapeutic results are shown in table 3, and the survival data are given in table 4. There was no statistically significant difference in response rate or survival in the two arms of this study. In the CAM group, the median survival time (MST) was 47 weeks for stable and responding patients and 10 weeks for patients with progressive disease. In the single-agent group, the MST was 56 weeks for stable and responding patients and 14 weeks for patients with progressive disease. The overall curve and the survival curves by response are shown in figures 2 and 3, respectively. There was a statistically significant survival advantage for responders and patients with stable disease versus those with progressive disease ($P < 0.0001$), and for responders versus patients with stable disease ($P = 0.002$). The relationship of response to histopathology is shown in table 5. There was no statistically significant advantage for either arm when histologies were compared.

The median duration of remission for the 11 responders receiving CAM was 30 weeks. There were only five objective responses to single-agent sequential therapy (four with cyclophosphamide and one with doxorubicin), with a median duration of 35 weeks. Thirty patients received second-line therapy with doxorubicin, with only a single partial remission of 15 weeks' duration. Only 11 patients received third-line therapy with methotrexate (no response), because most patients were not candidates for further therapy by the time their disease had progressed during the two prior chemotherapeutic trials. There are six patients still alive with CAM therapy (74+ to 104+ weeks from initiation of therapy), one of whom remains in continuous PR for 78+ weeks. There are six patients who remain alive with single-agent sequential therapy (58+ to 179+ weeks). One patient remains in continuous PR for 3+ years and has not received cyclophosphamide for 2 years, and one remains in CR for $2^1/_2$ years. Thus, despite overall low response rates, there are individual patients in both arms who derived significant benefit from therapy.

The survival curves for limited and extensive disease are shown in figure 4. There was a statistically significant survival advantage for patients with limited disease ($P = 0.02$).

DISCUSSION

During the past 5 years, combination chemotherapy regimens have become increasingly popular in the treatment of inoperable NSCLC. Numerous programs utilizing cyclophosphamide and doxorubicin have yielded impressive objective response rates of 30%–50%, with median survival times of 6–9 months (1–3); updated results from the same institutions confirmed an apparent therapeutic superiority over older chemotherapy programs (7,8). To substantiate claims for an improvement in survival in NSCLC, reference is frequently made to the 3–5-month MSTs with supportive care alone in Veterans Administration Lung Study Group (VALSG) protocols of the 1960s (9) and with BACON (bleomycin, doxorubicin, lomustine, vincristine, and mechlorethamine) in the early 1970s (10). However, the VALSG protocols excluded nobody, and the BACON regimen was also used to treat all patients regardless of KPS; in fact, 11 of 38 patients (29%) with extensive disease

TABLE 3.—Therapeutic results

	Sequential single agents	CAM	*P* value
% CR + PR	9* (1 CR)	22 (2 CR)	0.16
Median duration of remission in wks (range)	35 (15–170+)	30 (10–78+)	0.36
% stable disease	36	39	0.96
% progression	55	39	0.23

*4 patients receiving cyclophosphamide, 1 receiving doxorubicin (15 wks).

TABLE 4.—Survival results

	Sequential single agents	CAM	*P* value
Median survival in wks (range)	25 (4–179+)	32 (3–116)	0.24
% 1-yr survival	29	33	0.28
% 18-mo survival	12	20	0.42
% 2-yr survival	6	4	0.97

had a KPS of 10–40, and only one patient had a KPS of 80–100. The KPS is probably the major prognostic variable affecting survival in NSCLC. All of the recent combination chemotherapy regimens exclude patients with KPS < 50. In fact, one recently published paper excluded patients with KPS of 10–50, age > 65 years, prior chemotherapy, or creatinine clearance < 65 ml/minute (11). Interpretation of therapeutic advantage compared to historical controls must be viewed with caution, if not skepticism.

Use of the CAP-I combination regimen (cyclophosphamide, doxorubicin, and cisplatin) has been duplicated by the Southeastern Cancer Study Group (SECSG) (12). There were only seven objective responses in 91 patients (8%); however, the MST of 6 months was identical to that found in the original CAP-I study by Eagan et al (1). MACC (methotrexate, doxorubicin, cyclophosphamide, and lomustine) was utilized by the Eastern Cooperative Oncology Group (ECOG), and only five of 43 patients (12%) responded, with an MST of 15 weeks (13), in contrast to the 44% response rate and MST of 33 weeks in studies in NSCLC by Chahinian et al (3,8). CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) was also studied by ECOG (14), and they achieved only a 22% response rate and an MST of 19 weeks, compared to the original CAMP studies that achieved a 36% response rate and an MST of 36 weeks (2,7).

The results of the present study compared CAM to single-agent cyclophosphamide in 104 patients. Patients who had disease progression while receiving cyclophosphamide were then switched to doxorubicin and then to methotrexate after further progression. All patients had a minimal follow-up of 1 year. There was no statistically significant survival advantage for CAM, but results in both arms were statistically superior to

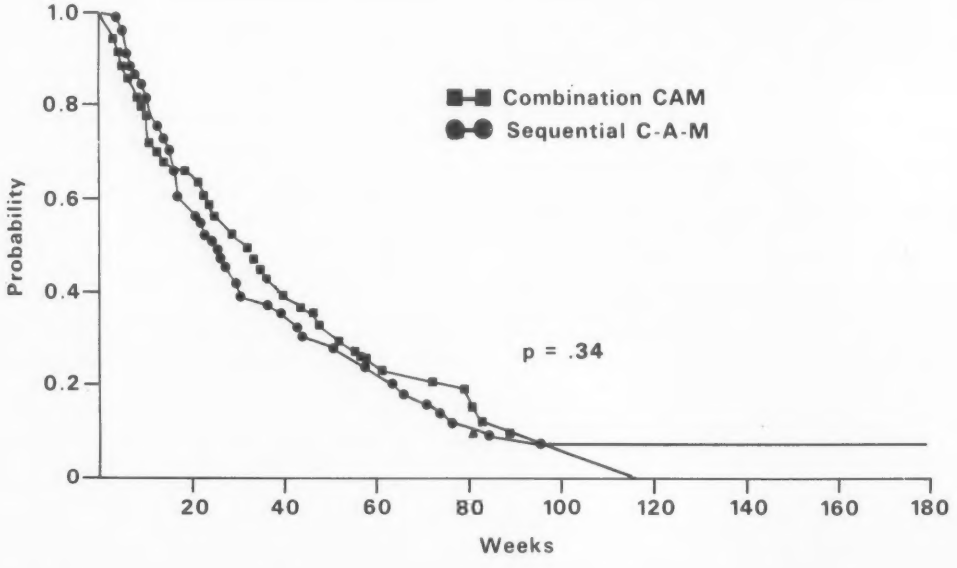

FIGURE 2.—Survival curves: CAM vs sequential single-agent cyclophosphamide, doxorubicin, and methotrexate.

TABLE 5.—Results by histology

Histology	Sequential single agents		CAM	
	Response*	MST (wks)	Response*	MST (wks)
Adenocarcinoma	1/19 (5)	26	2/13 (15)	34
Squamous cell carcinoma	2/22 (9)	19	8/35 (23)	28
Large cell carcinoma	2/12 (16)	40	1/3 (33)	46

*Values = No. of patients responding/No. in group (%).

those in VALSG historical controls, probably because of selection factors such as exclusion of patients with low KPS.

Although cyclophosphamide and doxorubicin have been incorporated in many combination chemotherapy regimens, they are probably, at best, marginally active agents. The Working Party for Lung Cancer compared COMB (cyclophosphamide, vincristine, semustine, and bleomycin) to single-agent cyclophosphamide in a random prospective study (15). Patient entry required histology of squamous cell carcinoma of the lung, no prior chemotherapy, serum creatinine < 1.6 mg/dl, and a Zubrod performance status < 4. Cyclophosphamide was given at a dose of 1100 mg/m^2 every 3 weeks to 27 eligible patients; there was only one objective response, and the MST was 14.4 weeks. Only one of 20 patients (5%) responded to COMB, and the MST on that arm was 10.9 weeks. The SECSG treated 91 patients with cyclophosphamide (500 mg/m^2) plus doxorubicin (50 mg/m^2) every 3 weeks (12). Eligibility requirements included creatinine clearance > 60 ml/minute and a KPS ≥ 50. Only three patients (3%) responded; however, in this random prospective study, the MST of 6 months and the overall survival curve were identical to those for CAP-I (12).

Another random prospective study compared doxorubicin (40 mg/m^2) plus cyclophosphamide (700 mg/m^2) every 3 weeks to single-agent vindesine (D. Luedke, personal communication). A KPS > 50 and no prior chemotherapy were the eligibility criteria. The objective response rate was only 9% for doxorubicin plus cyclophosphamide versus 22% for vindesine alone in 59 evaluable patients.

Pannuti et al (16) compared cyclophosphamide (1000 mg/m^2) every 3 weeks to cyclophosphamide, methotrexate, 5-FU, and hydroxyurea in 58 patients with no

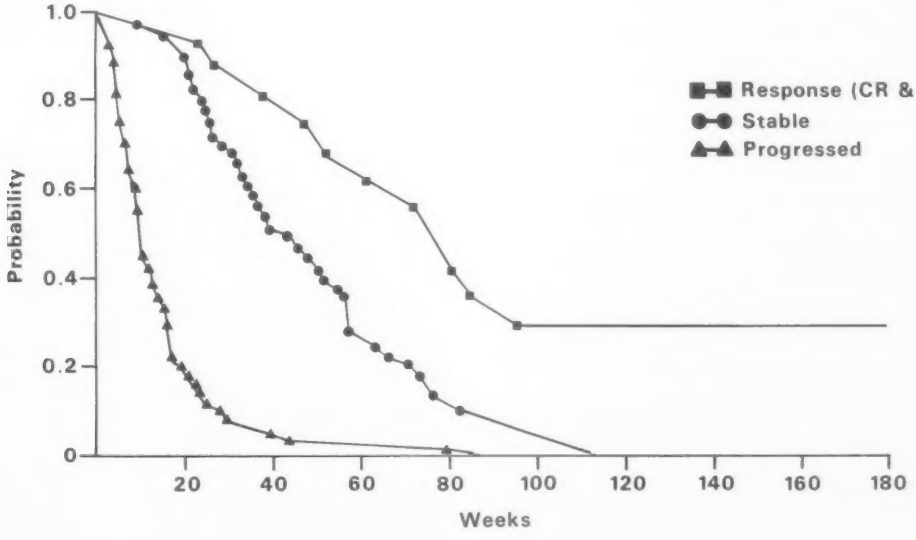

FIGURE 3.—Survival by response to therapy.

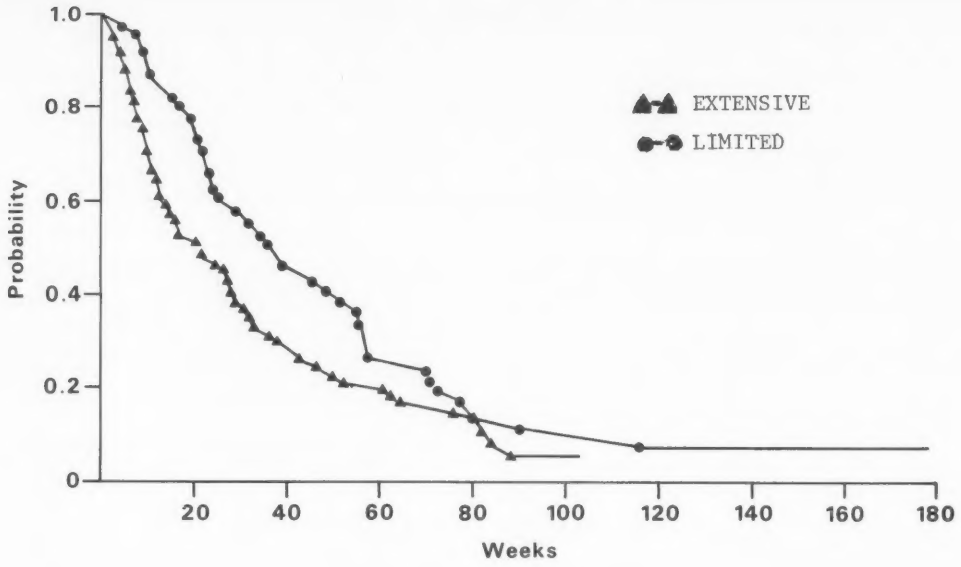

FIGURE 4.—Survival by extent of disease.

prior chemotherapy, KPS > 40, and age < 75 years. There was only one objective response in 29 patients (3%) treated with cyclophosphamide, with an MST of 24 weeks. The combination regimen had an identical 3% response rate with an MST of 26 weeks.

Davis et al (17) compared cyclophosphamide (600 mg/m^2) (41 patients) to cyclophosphamide plus lomustine and cyclophosphamide plus lomustine plus doxorubicin in 124 patients with extensive NSCLC, no prior chemotherapy, and KPS > 40. The response rates were only 5%, 8%, and 6%, respectively, with respective MSTs of 21, 17, and 19 weeks.

Finally, in our study, cyclophosphamide produced only four objective responses in 53 patients (8%). Even though doxorubicin was utilized as second-line therapy, it is difficult to be enthusiastic about its activity in NSCLC, since there was only one PR of 15 weeks' duration. Thus, these studies indicate that the objective response rate with cyclophosphamide is probably < 5% (eight of 179 responses), rather than the widely quoted 20%–30% response rate from the older literature.

Our major efforts in the past several years have been directed at utilizing phase II single agents as initial chemotherapy in inoperable NSCLC. Vindesine is thus far the only phase II agent we have tested that has significant single-agent activity. Six of 22 NSCLC patients (27%) with no prior chemotherapy achieved an objective response (18). The activity of vindesine is widely reproducible, with an overall objective response rate of 28% in 177 patients (18–23). However, more active agents will probably have to be found and utilized as components of a combination chemotherapy regimen before we can truly have an impact on survival in NSCLC. At Indiana University, we continue to evaluate phase II agents as initial chemotherapy for inoperable NSCLC.

REFERENCES

1. EAGAN RT, INGLE JN, FRYTAK S, ET AL. Platinum-based polychemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. Cancer Treat Rep 61:1339–1345, 1977.
2. BITRAN JD, DESSER RK, DEMEESTER TR, ET AL. Cyclophosphamide, adriamycin, methotrexate, and procarbazine (CAMP)—effective four-drug combination chemotherapy for metastatic non-oat cell bronchogenic carcinoma. Cancer Treat Rep 60:1225–1230, 1976.
3. CHAHINIAN AP, ARNOLD DJ, COHEN JM, ET AL. Chemotherapy for bronchogenic carcinoma: methotrexate, doxorubicin, cyclophosphamide, and lomustine (MACC). JAMA 237:2392–2396, 1977.
4. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
5. GEHAN EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203–223, 1965.
6. ARMITAGE P. Statistical Methods in Medical Research. Oxford, England, Blackwell Scientific Publications, 1971.
7. BITRAN JD, DESSER RK, DEMEESTER T, ET AL. Metastatic non-oat cell bronchogenic carcinoma. JAMA 240:2743–2746, 1978.
8. CHAHINIAN AP, MANDEL EM, HOLLAND JF, ET AL. MACC (methotrexate, adriamycin, cyclophosphamide, CCNU) in advanced lung cancer. Cancer 43:1590–1597, 1979.
9. GREEN RA, HUMPHREY E, CLOSE H, ET AL. Alkylating agents in bronchogenic carcinoma. Am J Med 46:516–525, 1969.

10. Livingston RB, Fee WH, Einhorn LH, et al. BACON (bleomycin, adriamycin, CCNU, Oncovin, and nitrogen mustard) in squamous cell lung cancer. Cancer 37:1237–1242, 1976.
11. Gralla RJ, Casper ES, Kelsen DP, et al. Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung: a randomized trial investigating two dosage schedules. Ann Intern Med 95:414–420, 1981.
12. Robert F, Birch R, Krauss S, et al. Randomized comparison of cyclophosphamide, adriamycin, and methotrexate with folinic acid vs. cyclophosphamide, adriamycin, and *cis*-platinum vs. cyclophosphamide plus adriamycin in advanced non-small cell lung cancer. Proc Am Assoc Cancer Res and ASCO 22:503, 1981.
13. Vogl SE, Mehta CR, and Cohen MH. MACC chemotherapy for adenocarcinoma and epidermoid carcinoma of the lung. Cancer 44:864–868, 1979.
14. Ruckdeschel JC, Mason B, Ettinger D, et al. Failure to define a "standard" chemotherapy for metastatic non-small cell bronchogenic carcinoma. Proc Am Assoc Cancer Res 23:144, 1982.
15. Bodey GP, Lagakos SW, Gutierrez AC, et al. Therapy of advanced squamous carcinoma of the lung. Cyclophosphamide versus "COMB." Cancer 39:1026–1031, 1977.
16. Pannuti F, Lelli G, Casadio M, et al. High-dose cyclophosphamide versus cyclophosphamide, methotrexate, 5-FU, and hydroxyurea (CMFH) in the treatment of stage III non-small cell bronchogenic carcinoma: a randomized trial. Cancer Treat Rep 64:1131–1134, 1980.
17. Davis S, Pandya MR, and Rambotti P. Single-agent and combination chemotherapy for extensive non-small cell carcinomas of the lung. Cancer Treat Rep 64:685–688, 1980.
18. Furnas B, Einhorn LH, and Rohn RJ. A phase II trial of vindesine (DVA) in non-small cell lung cancer (NSCLC). Proc Am Assoc Cancer Res and ASCO 21:448, 1980.
19. Gralla RJ, Raphael BG, Golbey RB, et al. Phase II evaluation of vindesine in patients with non-small cell carcinoma of the lung. Cancer Treat Rep 63:1343–1346, 1979.
20. Mattson K, Holsti LR, Salmo M, et al. Vindesine in the treatment of small cell and non-small cell bronchogenic carcinoma: preliminary results. Cancer Treat Rev 7:65–70, 1980.
21. Valdivieso M. Phase I and II studies of vindesine. Cancer Treat Rev 7:31–37, 1980.
22. Osterlind K, Dombernowsky P, Horbos S, et al. Vindesine as a single agent treatment of adeno-, large cell, and squamous carcinoma of the lung. *In* Contributions in Oncology. Munich, Karger-Verlag, 1981.
23. Hong WK, Rennacchio J, Krikorian J, et al. Prospective randomized trial of vindesine (DVA) vs. DVA with *cis*-platinum (DDP) in metastatic non-small cell lung cancer (NSCLC). *In* Contributions in Oncology. Munich, Karger-Verlag, 1981.

Cisplatin Therapy in Recurrent Childhood Brain Tumors[1]

Atiya B. Khan,[2,*] Bernard J. D'Souza,[2] Moody D. Wharam,[2] Lorraine A. A. Champion,[3] Lucius F. Sinks,[4] Shiao Y. Woo,[4] David C. McCullough,[4] and Brigid G. Leventhal[2,5]

Twenty-two patients with brain tumors were initially treated with surgery, radiation therapy, and/or adjuvant chemotherapy. Histologic diagnosis included 11 gliomas, seven ependymomas, one dysgerminoma, one teratocarcinoma, one pinealoma, and one small cell tumor. At recurrence, documented on computerized tomographic scan, cisplatin (60 mg/m^2/day iv × 2) was given every 3–4 weeks. Seventeen patients received two or more courses and were considered to be evaluable for response. Response parameters included change in the size of tumor on computerized tomographic scan and/or in clinical findings. Four patients had complete response, five had partial response, four had stable disease, and four had disease progression. Toxic effects were manageable. The number of patients with grade 3–4 toxic reactions, by category, were: thrombocytic, ten; leukocytic, four; renal, five; metabolic, four; gastrointestinal, none; and neurologic, seven. Two of the 14 patients tested had grade 3–4 ototoxicity. Acute fluid retention with decreased serum electrolytes and serious but reversible changes in the mental status, which were experienced in earlier patients, decreased in severity with increasing experience of the investigator. In summary, cisplatin appears to be active in a spectrum of brain tumors and should be studied further for therapeutic efficacy. [Cancer Treat Rep 66:2013–2020, 1982]

The current treatment for recurrent brain tumors poses a challenging therapeutic dilemma. Various chemotherapeutic regimens have produced varying degrees of response and often are only palliative. Cisplatin is a chelated heavy metal with proven antitumor activity in a wide spectrum of animal and human tumors (1–5). This report reviews the effectiveness and toxicity of cisplatin in recurrent brain tumors.

METHODS

The study group included 22 patients (11 from Johns Hopkins Hospital, ten from Georgetown University Hospital, and one from D. C. Children's Hospital). The histologic diagnoses were 11 gliomas (all grade 3 and 4), seven ependymomas, one dysgerminoma, one teratocarcinoma, one pinealoma, and one temporal lobe small cell tumor. Ages ranged from 4 to 22 years.

Patients were initially treated with surgery and radiotherapy (except for one patient), with or without adjuvant chemotherapy. Tumor recurrence was suggested by clinical deterioration and confirmed on computerized tomographic (CT) scan by either a new lesion (seven patients) or an increase in size of the pre-existing lesion (15 patients). Tumors recurred at the original site in all but six patients. Eight patients received additional radiotherapy and/or chemotherapy at the time of first recurrence but had either failed to respond or had tumor recurrence again before cisplatin was given (table 1).

Prior to initiation of therapy, all patients had a general physical and neurologic examination. Pretreatment laboratory studies included a cbc with differential and platelet counts, serum electrolytes, BUN, serum creatinine, 24-hour creatinine clearance, liver function tests, and an ECG. These studies, with the exception of the

[1]Received Sept 2, 1981; revised Sept 9, 1982; accepted Sept 27, 1982.

[2]Departments of Pediatric Oncology, Neurology, and Radiation Therapy, The Johns Hopkins University, Baltimore, MD.

[3]Oakland Children's Hospital and Medical Center, CA.

[4]Department of Pediatric Oncology, Georgetown University Hospital, Washington, DC.

[5]We thank Ms. Carolyn Jones for her assistance in the typing and preparation of this manuscript.

*_Reprint requests to:_ Atiya B. Khan, MD, Department of Pediatric Oncology, The Johns Hopkins Oncology Center, Rm 3-121, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—Treatment and response among 22 patients with recurrent brain tumors*

Patient No.	Histologic diagnosis	Initial therapy			Disease recurrence		Therapy for recurrence prior to cisplatin	Time between last therapy and start of cisplatin	No. of cisplatin courses	Response/ duration (mos)†
		Surgery	RT	Chemotherapy	Time	Site				
1	Glioblastoma, right parietal temporal lobe	Partial resection	5500 R	BCNU	5 mos	Local	None	1 mo	3	PR/6
2	Glioblastoma multiforme, right parieto-occipital region	Partial resection	5000 R	BCNU	6 mos	Local	PCNU × 3	4 mos	4	PR/5
3	Pontine-cerebellar astrocytoma	Biopsy	5000 R	None	7 1/2 yrs	Cerebellum	Drainage of cyst and biopsy of underlying nodule	19 mos	8	SD/41
4	Thalamic glioma, grade 3	Partial resection	None	BCNU 14 mos	3 yrs	Local	VP shunt + 6000 R	7 mos	8	SD/41
5	Glioblastoma multiforme, thalamic region	Gross total resection	6000 R	VCR Pred CCNU 1 yr	2 1/2 yrs	Local	None	35 mos	8	SD/24
6	Optic tract astrocytoma	Partial resection and shunt	5220 R	—	3 yrs		None	35 mos	1	NE/—
7	Mixed glioma, parieto-occipital, grade 3	Partial resection	5500 R	None	6 yrs	Local	Partial resection	6 yrs	2	PD/—
8	Pontine glioma	None	5500 R	None	6 mos	Local	—	6 mos	1	NE/—
9	Brain stem glioma	Biopsy	4500 R	—	1 1/2 yrs	Right frontal area	2500 R	8 mos	1	NE/—
10	Glioblastoma multiforme, right temporoparietal	Biopsy	5500 R	—	2 mos	Local	Partial resection	5 mos	2	PD/—
11	Glioblastoma multiforme, left cerebral hemisphere	Partial resection	5000 R	—	?	Local	—	1 mo	1	NE/—
12	Ependymoma, right occipital	Partial resection	6000 R	—	2 yrs	Gross total resection	CCNU	10 mos	3	PD/—
					4 yrs	Partial resection	VCR			
					4 1/2 yrs	Gross total resection	Procarb			
					5 1/4 yrs	VP shunt				
13	Ependymoma, 4th ventricle	Partial resection + VP shunt	5000 R to brain, 3000 R to spine	MOPP 6 mos	1 yr	Local	Iv MTX	5 mos	1	NE/—

14	Ependymoma, 4th ventricle	Partial resection + VA shunt	5500 R	MOPP 6 mos	1 yr	Local	None	1 mo	7	SD/11
15	Ependymoma, 4th ventricle	Gross total resection	5500 R	None	1 yr	Cerebellum, lumbar spinal cord	RT to spine	14 mos	9	CR/10
16	Ependymoblastoma, 4th ventricle	Partial resection	5500 R + spinal RT	VCR $1^1/_2$ yrs	3 yrs		—	13 mos	2	PD/—
17	Ependymoma, 4th ventricle	Gross total resection	5100 R + spinal RT	Iv MTX + BCNU	1 yr	Pineal	Subdurals drained bilaterally	1 mo	8	CR/41
18	Ependymoma, 4th ventricle	Gross total resection	6000 R	VCR CCNU 18 mos	2 yrs	Local	—	1 mo	3	PR/3
19	Suprasellar dysgerminoma	Partial resection	5000 R	—	2 yrs		Partial resection + 4140 R	11 mos	7	CR/10
20	Small cell tumor, left temporal lobe	Gross total resection	5000 R	VCR 9 mos	1 yr	Right parietotemporal mass + spinal cord (C7-T3)	MOPP 3 mos (VCR 1 yr)	2 mos	6	PR/6
21	Pineal teratocarcinoma	Partial resection	5000 R	None	1 mo	Local	—	1 mo	6	PR/5
22	Pinealoma	Gross total resection	4500 R	VCR CCNU	4 mos	Right frontal	—	1 mo	2	CR/3

*VP = ventriculoperitoneal; VA = ventriculoatrial; RT = radiotherapy; BCNU = carmustine; VCR = vincristine; Pred = prednisone; CCNU = lomustine; MOPP = mechlorethamine, vincristine, procarbazine, and prednisone; MTX = methotrexate; Procarb = procarbazine; SD = stable disease; NE = not evaluable; PD = progressive disease.

†Duration of response from beginning of cisplatin treatment.

ECG, were repeated prior to every course. CT scan and hearing tests were done every 2–3 months. Informed consent was obtained from all patients.

All patients were hospitalized for treatment, which usually required a 3–4-day admission, with hydration and baseline toxicity studies on Day 1 and administration of medication on 2 subsequent days. Thorazine was administered 12 hours prior to cisplatin therapy and every 8 hours to alleviate nausea and vomiting. Iv hydration with 3000 ml/m^2/day of 5% dextrose in 0.45 normal saline plus 20 mEq/L of KCl was started 6 hours prior to cisplatin therapy. Cisplatin was administered iv at a dose of 60 mg/m^2/day × 2 every 3–4 weeks. Each vial (10 mg) was reconstituted with 10 ml of sterile water to a concentration of 1 mg/ml and was infused over 4–6 hours. The drug was protected from light to prevent decomposition. Iv hydration was continued with drug administration and for 14 hours after the cisplatin infusion was complete. Patients were closely monitored for signs of fluid and electrolyte imbalance. In an attempt to diminish toxicity, cisplatin was initially administered with mannitol and/or Lasix, but these drugs were not routinely given in later patients. Serum sodium and urine output were carefully monitored. Iv fluid administration was adjusted to maintain a serum sodium level > 135 mEq/L.

Steroid and anticonvulsant dosages were not altered during therapy. Nephrotoxic antibiotics such as tobramycin and gentamicin were avoided. Therapy was discontinued with evidence of progressive disease after two courses or if severe renal side effects or ototoxicity developed. Between courses, patients did not experience drug-related limitation of activity.

Response Criteria

Two parameters were used to evaluate response: (*a*) decrease in the size and degree of enhancement produced by tumor on CT scan as compared to CT scans before therapy with cisplatin, and (*b*) improvement in documented neurologic deficits.

Complete response (CR) was defined as the disappearance of any evidence of tumor on CT scan, with resolution of all neurologic deficits that were present before cisplatin therapy. Partial response (PR) was defined as a > 50% reduction in tumor size on CT, with resolution of some neurologic deficits. Stable disease was defined as no improvement in CT scan or neurologic status, but no progressive worsening documented on either examination. Progressive disease was defined as an increase in size of enhancing lesions on CT scan and/or deterioration in neurologic signs and symptoms.

Toxicity Criteria

Neurologic, gastrointestinal, hematologic, and renal toxic effects were graded according to the criteria set forth by the Cancer and Leukemia Group B. Criteria for metabolic toxicity and ototoxicity grading were devised on our own (table 2).

RESULTS

Among the 22 patients, five received only one course of therapy because of rapid clinical deterioration and could not be adequately evaluated for response. Four patients had a CR, five had a PR, four had stable disease, and four had progressive disease. The duration of response is defined by duration of clinical and/or CT scan improvement and ranged from 3 to 41 months (mean, 14). In all patients who had a positive response, improvement in the clinical neurologic examination preceded improvement on CT scan. A CR was observed in four patients after two to eight courses (median, seven). A PR on CT scan was observed in five patients after two to five courses (median, two). Significant neurologic improvement was observed in eight patients after one to five courses (median, two), and return to normal neurologic status was observed in five patients after three to eight courses (median, five).

TABLE 2.—Grading of metabolic toxicity and ototoxicity

Grade	Metabolic (mEq/L)		Ototoxicity
	Serum K	Serum Na	
0: No abnormality	3.5	135	No toxicity
1: Mild	3–3.4	130–134	Tinnitus
2: Moderate	2.5–2.9	125–129	High frequency hearing loss
3: Severe	2.0–2.4	120–124	Low frequency hearing loss (helped with a hearing aid)
4: Maximal/life-threatening	2	120	Profound deafness

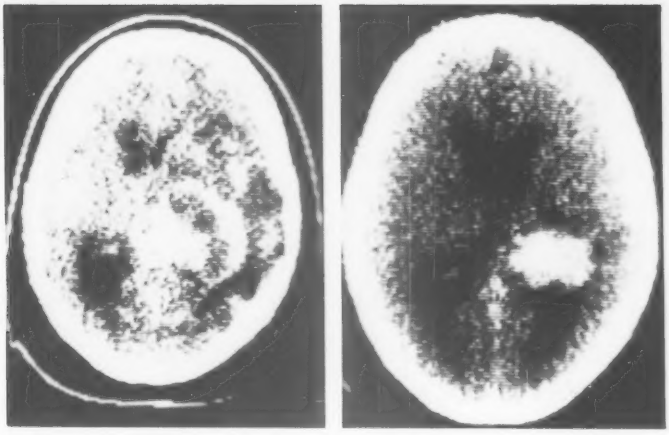

FIGURE 1.—PR of a glioblastoma to cisplatin in Patient 1. Scan on the left was obtained at recurrence, before cisplatin therapy. Scan on the right shows PR to therapy after 2 courses of cisplatin.

In the group of seven evaluable patients with gliomas, four tumors were located in the cerebral hemisphere, two in the thalamus, and one in the brain stem. None of these patients had a CR, but two (Nos. 1 and 2) had a PR (fig 1). Three patients (Nos. 3–5) had stabilization of disease and are alive 2–3$^1/_2$ years after treatment began. Two patients had progressive disease.

Four of the six evaluable patients with ependymomas had a favorable response. Two patients (Nos. 15 and 17) had a CR and are disease-free after nine and eight courses of treatment, at 1 and 3$^1/_2$ years after initiation of therapy (fig 2). Another patient (No. 18) showed definite improvement in the neurologic examination and on CT scan after two courses, but therapy had to be discontinued after three courses secondary to severe thrombocytopenia. This patient then had progressive disease and died. One patient (No. 14) has had stable disease for 11 months.

The remaining four patients with histologically small cell tumors (one pinealoma, one teratocarcinoma, one dysgerminoma, and one small cell tumor) all responded. Two patients (Nos. 19 and 22) had a CR (fig 3). One of

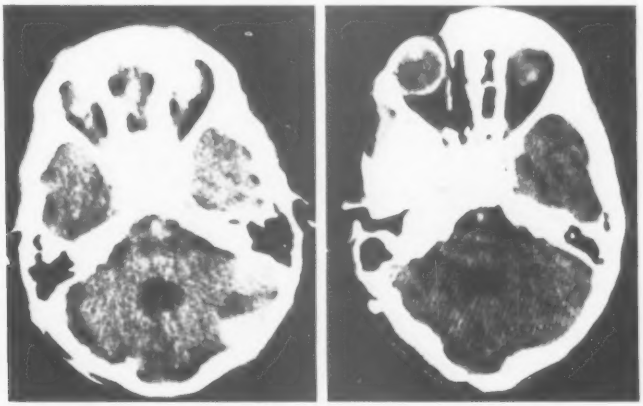

FIGURE 2.—CR in a patient with recurrent ependymoma. CT scan on the left shows recurrent tumors in the posterior fossa. Scan on the right shows CR after 6 courses of cisplatin.

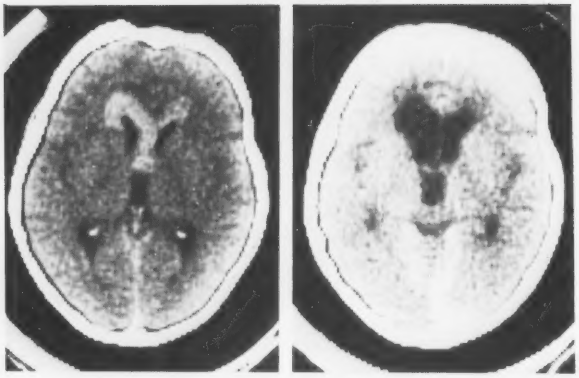

FIGURE 3.—CR of Patient 19. CT scan on the left shows paraventricular recurrence of suprasellar dysgerminoma. CT scan on the right is the appearance after 6 courses of cisplatin.

these patients (No. 19) later died of bilateral pneumonitis that was not biopsied, but lung cultures established at autopsy revealed *Staphylococcus aureus*. There was no evidence of tumor at postmortem examination. The other patient (No. 22), who showed a CR after two courses, developed renal toxic reactions necessitating discontinuation of treatment. This patient died following recurrence of her tumor. Two patients (Nos. 20 and 21) initially had a PR, but later had disease progression and were taken off therapy.

Five patients who died had autopsies. Two of these died while still responding. In both of these patients the autopsy findings confirmed those of the CT scan. One patient (No. 1) had a small 17-cm^3 tumor, which correlated with the 14-cm^3 volume calculated on the last CT scan (fig 1). The other patient (No. 19) had no tumor, but only necrosis around the lateral ventricles where tumor had previously enhanced. Three patients who were not evaluable for response were also autopsied. Two of these had progressive disease and one had hemorrhage in the tumor.

Toxicity

Table 3 documents the toxic effects seen in all 22 patients. Ten patients developed grade 3–4 platelet toxicity (< 75,000 cells/mm^3). One patient (No. 1) developed CNS bleeding 2 weeks after cisplatin administration. His platelet count was 11,000/mm^3 at that time. He did not have this toxic reaction with further treatment; perhaps initial sensitivity was due to a cumulative effect from his previous therapy with carmustine (BCNU). He improved with platelet transfusion and other supportive therapy. Four patients had

TABLE 3.—Toxic effects among 22 patients treated with cisplatin

	No. of patients by toxicity grade				
	0	1	2	3	4
Hematologic					
Platelets	8	1	3	3	7
Wbcs	10	3	5	2	2
Renal					
Creatinine	12	5	0	5	0
Metabolic	8	4	6	2	2
Gastrointestinal	0	10	12	0	0
CNS					
Hearing	2	0	10	2	0
Neurologic	15	0	0	5	2

severe granulocytopenia (< 1000 cells/mm^3). Two of these patients died: one of septicemia secondary to candida and klebsiella and one of salmonella sepsis. Patients who experienced moderate to severe hematologic toxicity early in the course of treatment had previously received other antineoplastic agents, and this could have contributed to the early onset of hematosuppression. Seven patients had also received spinal radiation, but there was no correlation between spinal radiation and early hematologic toxicity. Two deaths (Patients 1 and 19) secondary to pneumonia occurred at times when wbc counts were normal.

No patients developed grade 4 renal toxicity, but five had grade 3 (creatinine > 2 mg/100 ml or creatinine clearance < 75 mg/ml/min), necessitating discontinuation of treatment. None of the patients had grade 2 toxic reactions and five had grade 1. Twelve patients had normal renal function.

All patients experienced nausea and vomiting. None of the patients experienced grade 3–4 gastrointestinal toxicity, but 12 had grade 2.

Only 14 patients had serial hearing tests. Grade 2 ototoxicity was found in ten of these patients. Two patients had grade 3 hearing deficits requiring hearing aids. No ototoxicity was observed in two patients.

Of seven cases of grade 3–4 neurotoxicity, five were attributable to chemotherapy. Three patients (Nos. 6, 12, and 19) had seizures related to low serum sodium, which resolved with normalization of the serum sodium level. One patient (No. 1) had intracranial bleeding secondary to thrombocytopenia produced by cisplatin. This patient recovered with supportive treatment and did not have similar toxic reactions with repeated treatment. One patient (No. 9) developed respiratory difficulty and required mechanical support 24 hours after receiving cisplatin. This complication may have been due to the rebound effect of mannitol, which this patient had received with his cisplatin. He responded dramatically to steroids. Two other patients (Nos. 20 and 21) had a deterioration in their neurologic status during therapy, which was presumably related to progressive disease. The remaining 15 patients had no neurologic problems.

DISCUSSION

The results of our study indicate that cisplatin is active in a spectrum of brain tumors in children and is at least as promising as any other chemotherapeutic agents employed today. Past treatment of recurrent gliomas has resulted in a 40%–50% response rate with the following single agents: BCNU (6), lomustine (CCNU) (7), procarbazine (8), vincristine (9), teniposide (10), and high-dose methotrexate (11). Of our seven evaluable glioma patients, two showed PR, and three have had no progression of disease and are alive 2–3$^1/_2$ years after therapy (table 4). With ependymomas we have experienced even better results: two CR, one PR, and one stabilization of disease in six evaluable patients. Other drugs that have been used singly and in combination to treat ependymoma include vincristine, methotrexate (both intrathecal and systemic), BCNU, CCNU, procarbazine, and teniposide. Results in all these studies were similar, with response rates of 30%–50% (8–10,12–17).

All four evaluable patients from the miscellaneous tumor group had PR or CR. We are encouraged by the relatively high response rate to cisplatin in this group of tumors.

Gastrointestinal, renal, audiologic, hematologic, and neurologic toxic effects continue to be of major concern. Gastrointestinal toxicity, in particular nausea and vomiting, was the most distressing symptom to patients and necessitated the use of antiemetics with varying degrees of success. Robins et al (18) have demonstrated a safe and positive response with the combination of iv diazepam and prochlorperazine (Compazine) in 24 patients. This approach was not attempted in our study because of the high incidence of prochlorperazine reactions in children. Renal toxicity secondary to tubular damage is cumulative and seems to become evident after a total dose of 500 mg/m^2. In our patient population,

TABLE 4.—Summary of response to cisplatin

Tumor type	No. of evaluable patients	No. of patients with— CR	PR	Stable disease	Progressive disease	Duration of response (mos)
Glioma	7	0	2	3	2	5–41
Ependymoma	6	2	1	1	2	3–41
Pinealoma	1	1	0	0	0	3
Teratocarcinoma	1	0	1	0	0	5
Small cell tumor	1	0	1	0	0	6
Suprasellar dysgerminoma	1	1	0	0	0	10
Total	17	4	5	4	4	

ototoxicity secondary to cochlear damage was also cumulative and was first noted after 300 mg/m^2. In patients treated with cisplatin for other tumors, ototoxicity has not occurred this early. This early ototoxicity may be due to radiation therapy given to the brain in this group of patients. Two patients with severe hearing loss had significant improvement with use of a hearing aid. Recently, evidence for vestibular toxicity was reported in one patient (19). We had not performed vestibular function tests in our patients. Neurologic toxicity is probably related to fluid overload, hyponatremia, and hypomagnesemia and should be preventable. Clinical evidence for peripheral neuropathy was not observed in our study population. Long-term effects of this therapy are not known but are being assessed in the few patients who are still alive (> 1 year) after completion of therapy.

Although the number of patients treated in our study is small, these preliminary results suggest that cisplatin is an active agent in a spectrum of recurrent brain tumors. Stewart et al (20) measured cisplatin concentration in brain tissue, tumor tissue, and cerebrospinal fluid and reported that cisplatin selectively goes to tumor tissue, probably due to disruption of the blood-brain barrier. This selective uptake of platinum by tumor tissue is consistent with observed clinical effects. Additionally, cisplatin may prove to be more effective if used as an adjuvant to radiotherapy in view of the recent evidence that it may play a role as a radiosensitizer (21). The optimal duration of therapy and the long-term effects of cisplatin on normal CNS tissue have not been determined. Cumulative toxicity to the cochlea and the distal tubules of the kidney may well be the dose-limiting factor. An extended study using cisplatin in brain tumors is warranted.

REFERENCES

1. Rosenberg B, and Van Camp L. The successful regression of large solid Sarcoma 180 tumors by platinum compounds. Cancer Res 30:2799–2802, 1970.
2. Kociba RJ, Sleight SD, and Rosenberg B. Inhibition of Dunning ascitic leukemia and Walker 256 carcinosarcoma with *cis*-diamminedichloroplatinum (NSC-119875). Cancer Chemother Rep 54:325–328, 1970.
3. Highby D, Wallace HJ, Albert DJ, et al. Diamminodichloroplatinum. A phase I study showing responses in testicular and other tumors. Cancer 33:1219, 1974.
4. Kamalakar P, Freeman AI, Highby DJ, et al. Clinical response and toxicity with *cis*-dichlorodiammineplatinum (II) in children. Cancer Treat Rep 61:835–839, 1977.
5. Ochs JJ, Freeman AI, Chary KK, et al. Cis-DDP in osteosarcoma. American Cancer Society 19th Annual Meeting, Dec 1976.
6. Wilson CB, Boldrey EB, and Enot KJ. 1,3-Bis(2-chloroethyl)-1-nitrosourea (NSC-409962) in the treatment of brain tumors. Cancer Chemother Rep 54:273–281, 1970.
7. Fewer D, Wilson CB, Boldrey EB, et al. Phase II study of 1-(2-chloroethyl-)-3-cyclohexyl-1-nitrosoureas (CCNU; NSC-79037) in the treatment of brain tumors. Cancer Chemother Rep 56:421–427, 1972.
8. Kumar ARV, Renaudin J, Wilson CB, et al. Procarbazine hydrochloride in the treatment of brain tumors. Phase II study. J Neurosurg 40:365, 1974.
9. Fewer D, Wilson CB, Boldrey EB, et al. The chemotherapy of brain tumors. Clinical experience with carmustine (BCNU) and vincristine. JAMA 222:549, 1972.
10. Sklensky BC, Mann AF, Kaplan RS, et al. 4-Demethyl epipodophyllotoxin-D-thenylidene gludoside (PTG) in the treatment of malignant intracranial neoplasm. Cancer 33:460, 1974.
11. Rosen G, Ghavimi F, Nirenberg A, et al. High-dose methotrexate with citrovorum factor rescue for the treatment of central nervous system tumors in children. Cancer Treat Rep 61:681–690, 1977.
12. Lassman LP, Pearce GW, Banna M, et al. Vincristine sulphate in the treatment of skeletal metastases from cerebellar medulloblastoma. J Neurosurg 30:42, 1969.
13. Smart CR, Ottoman RE, Rochlin DB, et al. Clinical experience with vincristine (NSC-67574) in tumors of the central nervous system and other malignant diseases. Cancer Chemother Rep 52:733–741, 1968.
14. Newton WA, Jr, Sayers MP, and Samuels LP. Intrathecal methotrexate (NSC-740) therapy for brain tumors in children. Cancer Chemother Rep 52:257–261, 1968.
15. Walker MD, and Hurwitz BS. BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea; NSC-409962) in the treatment of malignant brain tumor—a preliminary report. Cancer Chemother Rep 54:263–271, 1970.
16. Ward HWC. CCNU in the treatment of recurrent medulloblastoma. Br Med J 1:642, 1974.
17. Shapiro WR. Malignant brain tumor chemotherapy: part II clinical studies. Clin Bull 3:2, 90, 1973.
18. Robins HI, Ershler WB, DeJongh L, et al. Antidiuretic effect of intravenous diazepam in patients receiving *cis*-diamminedichloroplatinum II: a pilot study. Med Pediatr Oncol 7:247–249, 1979.
19. Schaefer DS, Wright CG, Post JD, et al. Cis-platinum vestibular toxicity. Cancer 47:857–859, 1981.
20. Stewart DJ, Leavens M, Moore M, et al. Cis-DDP: human CNS distribution and use as a radiosensitizer in malignant brain tumors. Cancer Res 42:2474, 1982.
21. Douple EB, and Richmon RC. Platinum complexes as radiosensitizers of hypoxic mammalian cells. Br J Cancer 3:98, 1978.

Comparative Trial of Two Teniposide-Based Combination Chemotherapy Regimens for the Treatment of Advanced Malignant Lymphomas[1,2]

Michael J. O'Connell,* James Anderson, John M. Merrill, John M. Bennett, John H. Glick, Costan W. Berard, Richard S. Neiman, and Harvey S. Brodovsky[2]

One hundred and twenty-three patients with advanced measurable malignant lymphomas resistant to conventional chemotherapy were entered in a prospectively randomized trial of two teniposide (VM-26)-based combination chemotherapy regimens: V-PLAT (VM-26, cisplatin, and prednisone) and V-HEX (VM-26, hexamethylmelamine, and prednisone). Ninety-seven eligible and evaluable patients received protocol therapy. Sixteen percent of the patients had Hodgkin's disease, and 84% had non-Hodgkin's lymphoma. All patients were ambulatory (Eastern Cooperative Oncology Group performance status 0, 1, or 2), 70% had stage IV disease, 59% had "B" symptoms, and all had failed either two or three previous chemotherapy regimens. Toxicity was mainly hematologic and significantly greater with V-PLAT. Objective tumor responses were seen in nine of 45 patients (20%) treated with V-PLAT (duration, 4–35+ weeks) and in four of 51 patients (8%) treated with V-HEX (duration, 10–65+ weeks). Among the 12 patients with histologically confirmed histiocytic lymphoma treated with V-PLAT, five (42%) experienced objective tumor responses, including two complete responses. Overall median survival was approximately 6 months, with no difference between treatment regimens. Limited antitumor activity of these combination regimens in patients with advanced malignant lymphomas has been demonstrated. However, the objective response rates were not higher than we have previously seen with either VM-26 (22%) or hexamethylmelamine (27%) given in maximum tolerable doses as single agents. [Cancer Treat Rep 66:2021–2025, 1982]

Although chemotherapy has the well-documented potential for cure of disseminated malignant lymphomas, many patients will develop drug resistance to conventional chemotherapeutic agents. Thus, there is a need for non-cross-resistant chemotherapy regimens for patients with refractory lymphoma who have been treated with alkylating agents, vinca alkaloids, anthracyclines, and other commercially available cytotoxic drugs.

The Eastern Cooperative Oncology Group (ECOG) elected to study two teniposide (VM-26)-based combination chemotherapy regimens in patients with refractory lymphoma to assess their antitumor activity in patients previously exposed to systemic chemotherapy.

[1]Received May 19, 1982; revised Sept 13, 1982; accepted Sept 21, 1982.

[2]This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Mayo Clinic, Rochester, MN (*M. J. O'Connell*, CA-13650); Sidney Farber Cancer Institute, Boston (*J. Anderson*, CA-23318); Northwestern University Medical Center, Chicago, (*J. M. Merrill*, CA-17145); University of Rochester Cancer Center, NY (*J. M. Bennett*, CA-11083); Hospital of the University of Pennsylvania, Philadelphia (*J. H. Glick*, CA-15488); St. Jude Children's Research Hospital, Memphis (*C. W. Berard*); Mallory Institute of Pathology, Boston, (*R. S. Neiman*); and Jefferson Medical College, Philadelphia (*H. S. Brodovsky*, CA-14215). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia (CA-18281); Baystate Medical Center, Springfield, MA (CA-20182); Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland (CA-14548); Chicago Medical School, IL (CA-14144); Hahnemann Medical College, Philadelphia (CA-13611); Johns Hopkins Oncology Center, Baltimore (CA-16116); Medical College of Ohio, Toledo; University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, New York (CA-16395); Rush-Presbyterian-St. Luke's Medical Center, Chicago (CA-10948); University of Pretoria, South Africa (CA-21692); SUNY-Stony Brook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); Wisconsin Clinical Cancer Center, Madison (CA-21076); and Yale School of Medicine, New Haven, CT (CA-15485).

**Reprint requests to:* Michael J. O'Connell, MD, Department of Oncology, Mayo Clinic, Rochester, MN 55905.

The epipodophyllotoxin VM-26 was chosen based on previous ECOG experience (1) in patients with advanced lymphoma, which demonstrated objective tumor response in eight of 36 patients treated with VM-26 as a single agent, as well as on other reports in the literature of antitumor activity against malignant lymphomas (2–5).

Cisplatin was selected to be combined with VM-26 based on its reported single-agent activity in patients with advanced malignant lymphomas (6–8), its unique chemical structure which is quite dissimilar from other chemotherapeutic agents currently used for the treatment of malignant lymphomas, and its relative lack of myelosuppression. A pilot study of the VM-26, cisplatin, and prednisone regimen (V-PLAT) was carried out in nine patients at the Mayo Clinic to establish the appropriate dosage levels of VM-26 which could be combined with moderate-dose cisplatin (45 mg/m^2 given over 3 days) in previously treated lymphoma patients. Leukopenia (wbc count nadir $< 2000/mm^3$) and thrombocytopenia (platelet count nadir $< 100{,}000/mm^3$) were observed in four of the nine patients at a VM-26 dose of 25 mg/m^2 daily for 3 consecutive days in combination with cisplatin at a dose level of 15 mg/m^2 daily $\times$ 3. Prednisone was given at an empiric dose of 100 mg/m^2 for 5 consecutive days because of its known antitumor activity against malignant lymphomas and its palliative effects on the systemic symptoms which frequently occur in patients with advanced lymphomas.

Hexamethylmelamine was chosen for study based on previous ECOG experience (9) in which nine of 36 patients with advanced lymphomas obtained an objective remission when the drug was given as a single agent. The daily oral dose was decreased from 200 mg/m^2 $\times$ 21 days when given as a single agent to 150 mg/m^2 $\times$ 10 days when combined with VM-26 and prednisone in the VM-26, hexamethylmelamine, and prednisone regimen (V-HEX).

We used a randomized study design to minimize selection bias and to prospectively assess the relative antitumor efficacy of these two regimens in patients with advanced malignant lymphomas who had been previously treated with cytotoxic chemotherapy.

MATERIALS AND METHODS

All patients had a biopsy-proven diagnosis of malignant lymphoma of one of the following histologic types: Hodgkin's disease, nodular non-Hodgkin's lymphoma, or diffuse non-Hodgkin's lymphoma. Histologic diagnosis was confirmed by regional and/or national Panel For Lymphoma Clinical Trials review in 76% of the eligible patients. All patients had disseminated disease (stage III or IV according to the Ann Arbor classification) except one with progressive stage II disease following radiotherapy. Patients in a leukemic phase of their disease (as defined by the presence of circulating lymphoblasts in the blood) or > 75 years of age were not eligible for study. All patients had received at least one chemotherapy trial, with or without prior radiotherapy, and had failed or relapsed. Patients who had received any of the study agents, with the exception of prednisone, were excluded. All patients had at least one measurable indicator lesion to serve as an objective parameter of response to therapy. Radiographic findings were acceptable, provided clear-cut measurements could be made. Clearly defined defects measuring at least 5 cm in diameter on radionuclide, computed tomographic, or ultrasound examination of the liver were considered acceptable measurable parameters. Enlarged spleen and/or liver extending at least 5 cm below the costal margin on quiet respiration was also acceptable as a measurable parameter (biopsy proof of liver involvement by lymphoma was required if the liver was considered as the sole area of measurable disease). Patients had not received any cytotoxic chemotherapy or radiation therapy within 3 weeks of entry in the study (6 weeks for nitrosourea chemotherapy). Pretreatment laboratory test requirements included wbc count $> 4000/mm^3$, platelet count $> 100{,}000/mm^3$, total serum bilirubin < 2.0 mg/dl, and serum creatinine ≤ 1.5 mg/dl. Patients must have had an ECOG performance status of 0, 1, or 2. Patients with more advanced degrees of disease-related debility (performance status 3 or 4) were not eligible.

Patients were stratified according to histology (Hodgkin's disease, nodular non-Hodgkin's lymphoma, or diffuse non-Hodgkin's lymphoma) and performance status (ECOG performance status 0–1 or 2). They were then randomized to receive chemotherapy with either the V-PLAT or the V-HEX regimen.

VM-26 was given by slow iv infusion over a 30- to 40-minute period on Days 1, 2, and 3, and prednisone was given orally at a dose of 100 mg/m^2 daily on Days 1–5 in both regimens. In the V-PLAT regimen, cisplatin was given at a dose of 15 mg/m^2 on Days 1, 2, and 3. In the V-HEX regimen, hexamethylmelamine was given orally at a dose of 150 mg/m^2 daily on Days 1–10. Treatment was repeated every 28 days if the patient was stable or had experienced an objective tumor response. A provision was made for escalation of the VM-26 dose by 20% if no hematologic toxicity was observed from the preceding course of chemotherapy.

Patients were judged to have a complete response if there was complete regression of all palpable and X-ray-demonstrable disease. Partial response was indicated if there was at least a 50% reduction in the sum of the products of the perpendicular diameters of measurable lesions, or at least a 30% decrease in the linear measurements of liver or spleen. Furthermore, to qualify for either complete or partial response, there could be no decline in the performance status, no significant

clinical deterioration, and the observed duration of response had to last for at least 4 weeks. Increase in liver or spleen size by at least 2 cm, symptomatic deterioration due to malignant lymphoma, or a decline in performance status of more than one level not explained by other medical conditions was considered disease progression.

Differences in toxicity by treatment were assessed using Fisher's exact test (10). Survival from the start of the study was estimated using the method of Kaplan and Meier (11). Comparisons of survival were made using the log-rank test (12).

RESULTS

Administrative Data

One hundred and twenty-three patients were entered in the study. Six patients (three randomized to each treatment group) were cancelled prior to beginning therapy. Fourteen patients (11 V-PLAT and three V-HEX patients) were found on non-blinded review of the records not to meet eligibility criteria for the following reasons: unacceptable pretreatment laboratory value (six patients), inadequate interval from previous oncologic therapy (four), lack of measurable indicator lesion (two), unacceptable pretreatment performance status (one), and age > 75 years (one). Five additional patients were excluded on the basis of pathology review (two V-PLAT and three V-HEX patients), indicating an ineligible histologic diagnosis. No records were received for one patient randomized to V-PLAT. Thus, there were 97 patients (46 treated with V-PLAT and 51 treated with V-HEX) available for analysis. Characteristics of the eligible patients who received protocol therapy are summarized in table 1.

TABLE 1.—Patient characteristics

	No. of patients	
	V-PLAT	V-HEX
Eligible for evaluation	46	51
Age in yrs		
< 40	13	10
40–60	18	24
> 60	15	17
Sex		
Male	24	31
Female	22	20
Histology*		
Hodgkin's disease	8	8
Nodular lymphoma	8	10
Diffuse lymphoma	17	25
Nodular and diffuse lymphoma	12	7
Unclassifiable lymphoma	1	1
Anatomic stage†		
III	11	17
IV	35	33
Symptomatic stage†		
A	15	24
B	31	26
Unknown	—	1
Performance status		
0	14	12
1	21	25
2	11	14
Prior chemotherapy		
2 regimens	21	31
3 regimens	24	20
Unknown	1	—
Prior radiotherapy		
1 course	22	28
2 courses	10	12
3 courses	13	11
Unknown	1	—

*Non-Hodgkin's lymphomas classified according to the Rappaport classification. Specimens with focal nodularity *and* diffuse pattern listed as "nodular and diffuse."

†Ann Arbor classification. 1 patient treated with V-HEX had progressive stage II disease in a previously irradiated site.

Toxicity

Chemotherapy toxicity was primarily hematologic. Patients treated with the V-PLAT regimen experienced significantly greater hematologic toxic effects than did those treated with V-HEX. Among the 89 patients evaluable for hematologic toxicity, 31% treated with V-PLAT as compared to 6% treated with V-HEX had wbc count nadirs $< 2000/mm^3$ and/or platelet count nadirs $< 50,000/mm^3$ ($P < 0.01$). One patient treated with each regimen died of sepsis during a period of chemotherapy-induced leukopenia. A third patient (who was allergic to allopurinol) received treatment with V-HEX and died with acute renal failure and hyperuricemia, despite fluid administration and alkalinization, presumed to be secondary to tumor cell lysis. Six patients, all treated with the cisplatin-containing regimen (V-PLAT), experienced severe vomiting. Overall, treatment-related side effects were not considered to be excessive in this population of previously treated lymphoma patients.

Tumor Response

A summary of the characteristics of patients experiencing objective tumor response is shown in table 2. Patients experiencing mixed response or transient antitumor effects lasting < 4 weeks were classified as nonresponders and were excluded from this table. One patient treated with V-PLAT was not evaluable for tumor response because of lack of serial tumor measurements. The overall objective response rate was 14% (13 responses among 96 patients). More patients responded to V-PLAT (20%, nine responses among 45 patients) than to V-HEX (8%, four responses among 51

TABLE 2.—Characteristics of responding patients

Patient No.	Regimen	Age (yrs)	Sex	Histology*	Stage	Performance status	Response†	
							Type	Duration (wks)
1	V-PLAT	45	F	DH	IV-B	2	CR	12
2	V-PLAT	53	M	DH	IV-A	1	CR	35
3	V-PLAT	65	F	Composite (NPDL; DH)	IV-B	2	CR	35+
4	V-PLAT	48	M	DH	IV-B	1	PR	21
5	V-PLAT	59	M	Nodular + diffuse histiocytic	IV-B	1	PR	6
6	V-PLAT	48	F	Nodular + diffuse mixed	III-B	1	PR	10
7	V-PLAT	61	F	NPDL	IV-B	0	PR	4+
8	V-PLAT	20	M	HD	IV-B	0	PR	8
9	V-PLAT	53	F	NM	III-A	1	PR	18+
10	V-HEX	54	M	DWDL	IV-A	0	CR	65+
11	V-HEX	67	M	DWDL	IV-B	1	PR	10
12	V-HEX	16	M	DM	III-B	1	PR	17
13	V-HEx	53	F	Nodular + diffuse lymphocytic	III-B	0	PR	27+

*DH = diffuse histiocytic lymphoma; NPDL = nodular poorly differentiated lymphocytic lymphoma; DM = diffuse mixed lymphoma; DWDL = diffuse well-differentiated lymphocytic lymphoma; HD = Hodgkin's disease; NM = nodular mixed lymphoma.

†PR = partial response; CR = complete response. Duration of response measured from first documentation of response to disease progression.

patients). This difference was of marginal statistical significance ($P = 0.08$). When response to treatment was evaluated according to histology, it was apparent that neither treatment was effective for patients with Hodgkin's disease (one response among 16 patients). Overall, objective responses were seen in two of 18 patients (11%) with nodular non-Hodgkin's lymphoma, in seven of 42 patients (17%) with diffuse non-Hodgkin's lymphoma, and in three of 19 patients (16%) with non-Hodgkin's lymphoma which demonstrated both nodular and diffuse growth pattern. The patient subgroup in which the most striking antitumor effect was noted included patients with histiocytic lymphoma treated with V-PLAT. Among the 12 patients with histiocytic lymphoma treated with V-PLAT whose histologic diagnosis was confirmed by pathology review (nine with diffuse histiocytic and three with nodular and diffuse histiocytic), five patients (42%) had an objective tumor response, including two complete responses.

Survival

There was no significant difference in survival, measured from the start of therapy, between patients receiving either of the chemotherapy regimens (fig 1). Median survival was approximately 6 months for both patient groups.

Analysis of the various patient and disease characteristics listed in table 1 indicated a statistically significant relationship between patient survival and anatomic stage ($P = 0.01$), symptomatic stage ($P = 0.002$), and pretreatment performance status ($P = 0.06$). Those patients with extranodal disease (stage IV), "B" symptoms, or impaired performance status had shorter survival. Patients with diffuse non-Hodgkin's lymphoma had somewhat shorter survival than did those with nodular non-Hodgkin's lymphoma or Hodgkin's disease, but this difference was not statistically significant ($P = 0.15$).

DISCUSSION

This investigation has documented limited antitumor activity for the two chemotherapy regimens studied in previously treated patients with advanced malignant lymphoma. Although the objective response rate observed with the V-PLAT regimen (20%) was higher than that seen with the V-HEX regimen (8%), this difference was of borderline statistical significance. Furthermore, the V-PLAT regimen was associated with more hematologic and gastrointestinal toxicity, and there was no difference in patient survival between the regimens.

These results should be considered in the context of previous ECOG experience in the treatment of advanced lymphoma patients with VM-26 or hexamethylmelamine as single agents, in which objective response rates of 22% (1) and 25% (9), respectively, were observed. Thus, neither of the VM-26-based combination regimens as given in this study produced an objective response rate superior to results of our previous experience with single-agent therapy. The failure to produce higher response rates with the V-PLAT or V-HEX regimen may in part be explained by the dosage reduction of VM-26 which was required in previously treated lymphoma patients with limited bone marrow reserves.

The high degree of antitumor activity of the V-PLAT regimen in heavily previously treated patients with histiocytic lymphoma in the present study (42% objective

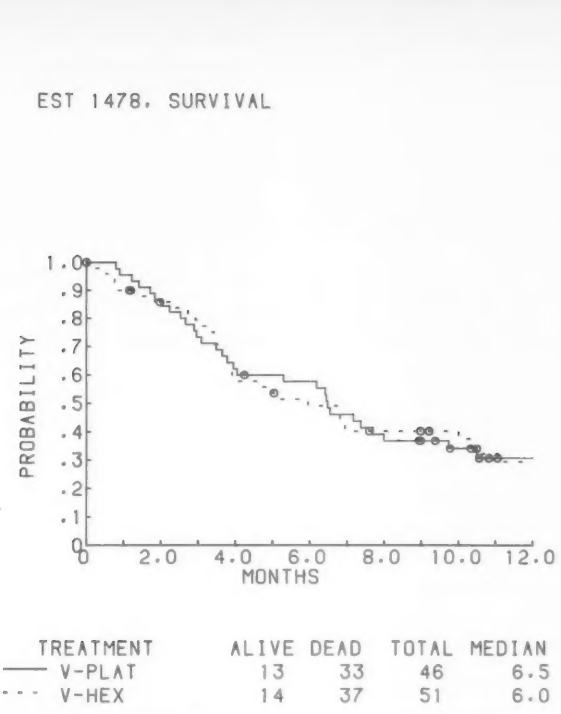

FIGURE 1.— Patient survival from initiation of protocol therapy, according to treatment regimen.

response rate among 12 patients) is of interest. In our previous study of VM-26 as a single agent in patients with refractory lymphoma (1), four of 12 patients (33%) with diffuse histiocytic lymphoma had objective responses, including two complete responses. Thus, VM-26 given alone or in combination with other agents may be particularly useful in the management of patients with histiocytic lymphoma.

The opportunity to more adequately evaluate the role of myelosuppressive agents such as VM-26 in the treatment of malignant lymphoma may require investigation in patients not previously treated with cytotoxic agents who would be expected to have more adequate bone marrow reserves. In addition, there would be considerable interest in further studies of nonmyelosuppressive agents in patients with refractory lymphomas to develop effective "second-line" regimens for previously treated patients.

REFERENCES

1. CHIUTEN DF, BENNETT JM, CREECH RH, ET AL. VM-26, a new anticancer drug with effectiveness in malignant lymphoma: an Eastern Cooperative Oncology Group study (EST 1474). Cancer Treat Rep 63:7–11, 1979.
2. DOMBERNOWSKI P, NISSEN NI, and LARSEN V. Clinical investigation of a new podophyllum derivative, epipodophyllotoxin, 4'-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
3. NISSEN NI, LARSEN V, PEDERSEN H, ET AL. Phase I clinical trial of a new antitumor agent, 4'-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 56:769–777, 1972.
4. SONNTAG RW, SENN HJ, NAGEL G, ET AL. Experience with 4'-demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-beta-D-glucopyranoside); VM-26; NSC-122819 in the treatment of malignant lymphosis. Eur J Cancer 10:93–98, 1974.
5. EUROPEAN ORGANIZATION FOR RESEARCH ON THE TREATMENT OF CANCER. COOPERATIVE GROUP FOR LEUKAEMIAS AND HAEMATOSARCOMAS. Clinical screening of epipodophyllotoxin VM-26 in malignant lymphomas and solid tumours. Br Med J 2:744–748, 1972.
6. TALLEY RW, O'BRYAN RM, GUTTERMAN JU, ET AL. Clinical evaluation of toxic effects of *cis*-diamminedichloroplatinum (NSC-119875)—phase I clinical study. Cancer Chemother Rep 57:465–471, 1973.
7. ROSSOF AH, SLAYTON RE, and PERLIA CP. Preliminary clinical experience with *cis*-diamminedichloroplatinum (II) (NSC 119875, CACP). Cancer 30:1451–1456, 1972.
8. GOTTLIEB JA, and DREWINKO B. Review of the current clinical status of platinum coordination complexes in cancer chemotherapy. Cancer Chemother Rep 59:621–628, 1975.
9. BENNETT JM, LENHARD RE, JR, EZDINLI E, ET AL. Chemotherapy of non-Hodgkin's lymphomas: Eastern Cooperative Oncology Group experience. Cancer Treat Rep 61:1079–1083, 1977.
10. COX DR. Analysis of binary data. London, Methuen, 1970.
11. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
12. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1–39, 1979.

Phase I Trial of *Staphylococcus aureus* Cowan I Immunoperfusion[1,2]

Gerald Messerschmidt,[3,*] Charles Bowles,[4] Danny Dean,[3] Margaret Parker,[5] Robert Lester,[5] Regina Dowling,[3] Thomas Holohan,[3] Louise Osborne,[3] B. Frank Schaff,[3] Kathleen McCormack,[4] Robert Corbitt,[4] Terrance Phillips,[6] Eli Glatstein,[7] and Albert Deisseroth,[3,8]

Staphylococcus aureus **Cowan I has shown antitumor activity in in vitro and in animal tumor models. It is hypothesized that this antineoplastic effect results from the interaction of protein A on the cell surface of Cowan I strain *S. aureus* and immunosuppressive circulating immune complexes. Therefore, we treated five patients with ex vivo plasma immunoperfusion over killed and fixed *S. aureus* Cowan I. Toxic effects were marked in all patients and appeared to be related to the plasma volume infused and rate of infusion. Toxic reactions occurred in the cardiovascular, respiratory, and hematopoietic systems. No responses, even minimal or transient, were observed in this phase I trial. This toxicity may be reduced if the rate of plasma infusion decreases. [Cancer Treat Rep 66:2027–2031, 1982]**

Immune complexes have been implicated as one of the circulating plasma factors capable of suppressing cellular immunity in cancer patients (1). Protein A has the unusual property of binding free immunoglobulins and immune complexes by one of two Fc receptors within the molecule (2). Steele et al (3) hypothesized that the immune suppression could be reversed by removing immunosuppressive immunoglobulins by protein A found on the cell wall of *Staphylococcus aureus*. Studies demonstrated that the original immunosuppressive tumor-bearing rat sera no longer contained this capacity after exposure to *S. aureus* in vitro (3). Bansal et al (4), using a similar hypothesis, treated a patient with colon carcinoma with an ex vivo immunoperfusion system containing *S. aureus*. Changes were observed in the immune system and there was a decrease in tumor measurements. Subsequent animal studies with spontaneous adenocarcinoma treated with *S. aureus* immunoperfusion have demonstrated objective tumor responses in approximately 50% of the animals (5,6). Our group, therefore, applied this therapy to patients with cancer in a phase I trial.

MATERIALS AND METHODS

Patients with malignancy that had previously been treated with irradiation or chemotherapy and who had no standard curative therapy available were eligible for the trial. All gave informed consent. The first patient was treated in the Apheresis Unit of the National Cancer Institute (NCI). All subsequent patients were treated in the Critical Care Medicine Department (NIH). Access to the circulatory system was obtained by arterial or venous punctures and whole blood was withdrawn and separated into plasma and cellular elements by continuous flow centrifugation (AMINCO, Silver Spring, MD). The cellular elements were returned to the patient and the plasma was diverted to pass over a cartridge filter (Gelman Scientific, Ann Arbor, MI) which contained *S. aureus* (Pansorbin; Calbiochem, La Jolla, CA) at a dose of 1.0 g/kg of patient body weight. This amount of *S. aureus* was distributed in two to 14 cartridges. Plasma was pumped through the filters at 25 ml/minute. All patients treated in the Critical Care Medicine Department had continuous arterial pressure

[1]Received June 24, 1982; revised Sept 13, 1982; accepted Sept 30, 1982.

[2]Presented in part at the American Society of Clinical Oncology meeting, Apr 26, 1982, St. Louis, MO.

[3]Experimental Hematology Section, Pediatric Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4]Hazelton Laboratories, Vienna, VA.

[5]Critical Care Medicine, Clinical Center, National Institutes of Health, Bethesda, MD.

[6]Department of Medicine, George Washington University, Washington, DC.

[7]Radiation Oncology Branch, National Cancer Institute, Bethesda, MD.

[8]*Present address:* Hematology and Oncology, San Francisco Veterans Medical Center, CA.

[*]*Reprint requests to:* Gerald L. Messerschmidt, MD, Medical Oncology and Hematology Section, Department of Medicine, Wilford Hall USAF Medical Center, Lackland Air Force Base, TX 78236.

TABLE 1.—Patient characteristics

Patient No.	Diagnosis	Age (yrs)	Performance status
1	Synovial cell carcinoma	28	4
2	Colon adenocarcinoma	65	3
3	Esophageal carcinoma	33	3
4	Malignant melanoma	56	3
5	Esophageal carcinoma	63	4

monitoring and Swan-Ganz catheter monitoring performed. The patients were treated no more than once a week with no more than 2 weeks between perfusion treatments. We attempted to perfuse 100% of the calculated plasma volume. The plasma volume was calculated by the following equation: calculated plasma volume = (88 ml/kg) (100 – hematocrit) (body weight in kg). The plasma was anticoagulated with preservative-free heparin.

RESULTS

Five patients were treated. The patient characteristics prior to therapy are presented in table 1. Each patient had multiple courses of irradiation and chemotherapy prior to this trial. Patients 1 and 5 had severe pulmonary compromise as a result of tumor.

Eight immunoperfusion treatments were performed in these five patients. Table 2 outlines the procedure characteristics. We attempted to pass 100% of the calculated plasma volume through the cartridge filter containing the *S. aureus*. Because of severe toxicity and technical problems, only 24%–60% of the desired amount of plasma was perfused and returned to the patients. The technical problem that occurred with the cartridge filter system was filter plugging, necessitating manual pressure to pass the plasma through and return it to the patient. This occurred at some time with every procedure. Because of this technical problem and the toxicity, the plasma pump was turned on and off several times. Thus, the average plasma flow rate was less than that in the design, ranging from 3.4 to 15 ml/minute (table 2).

Toxicity

The toxicity of the procedure was excessive and resulted in termination of the protocol after the eighth perfusion procedure. Toxicity was manifested in the cardiovascular, respiratory, and hematologic systems. Figures 1–3 demonstrate the cardiovascular toxicity. Few changes were observed during the initial 100 ml of plasma infused. Following this amount, cardiac output and index increased in all patients but one (fig 1, A and B). Concomitant with this increase in cardiac function was an associated increase in mean arterial blood pressure (fig 2). The cardiac index and output remained elevated until the procedure was terminated and slowly returned to baseline by 8–12 hours. Mean arterial blood pressure began to fall as approximately 300 ml of perfused plasma was reinfused (fig 2), even as the cardiac output remained elevated. The fall in mean arterial pressure corresponded to a decrease in systemic vascular resistance (fig 3). Fluid administration was required in all eight procedures to maintain the mean blood pressure > 60 mm Hg. Vasoconstrictors were necessary in six of the eight procedures to maintain this level.

Because of the profound hypotension, the plasma pumps were turned off frequently. When the plasma reinfusion was interrupted, mean arterial blood pressure began to rise within 3–8 minutes. However, as the reinfusion was begun again, mean blood pressure fell again.

Respiratory rate increased by 25%–110% in these procedures. This was accompanied by a decrease in PaO_2 and $PaCO_2$ (fig 4, A and B). Two patients arrested and

TABLE 2.—Procedure characteristics with *S. aureus* immunoperfusion

Patient No.	Weight (kg)	Calculated plasma volume (L)	Plasma perfused and returned		Time (mins)	Average plasma flow rate (ml/min)
			Total ml	%		
1	60	3.5	1300	37	95	13.7
1	60	3.5	1003	29	75	13.4
2	54	3.3	1611	49	150	10.7
3	99	5.9	3517	60	235	15.0
3	99	5.9	2754	47	330	8.3
4	50	2.9	826	29	150	5.5
4	50	2.9	686	24	200	3.4
5	74	4.6	1190	26	90	13.2

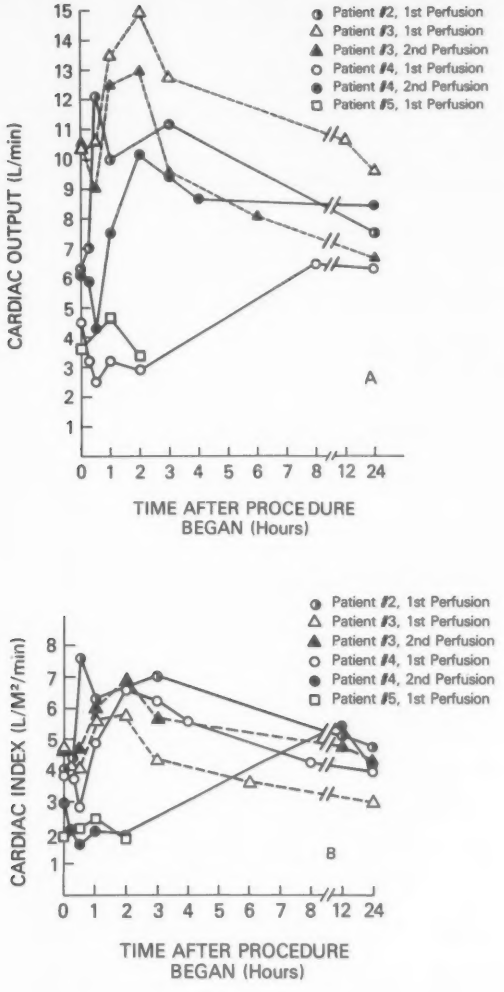

FIGURE 1.—A. Cardiac output. B. Cardiac index, in the 6 patient procedures performed in the Critical Care Department.

died as a result of respiratory problems. Both patients (Nos. 1 and 5) had severe pulmonary compromise prior to treatment. The first patient had approximately 75% of his chest volume obliterated by tumor and inaccessible fluid. Prior to his second procedure he had a PaO_2 of 54 mm Hg. At the end of the procedure, despite oxygen by mask, a PaO_2 of 29 mm Hg was measured, and he arrested and died. The fifth patient had esophageal carcinoma surrounding his trachea, resulting in severe extrathoracic airway obstruction. During this first procedure his respiratory rate increased to 44. As a result, he could not adequately ventilate, and he arrested and died. Autopsies performed on both patients failed to show any evidence of tumor response or to indicate the etiology of the cardiopulmonary response.

Hematopoietic toxicity was manifested in the total wbc and platelet counts. Total wbc counts decreased by 4 hours after the initiation of the procedure (fig 5A). By 8 hours, the total wbc count had returned to baseline or above. The decrease in total wbc count occurred as a resultant decrease in all cells, ie, granulocytes and lymphocytes. If an elevation over baseline occurred, this was most commonly associated with an increase in granulocytes and band forms. A normal differential count was observed by 48 hours. Platelet counts also decreased dramatically (fig 5B), again by 4 hours. However, unlike the total wbc count, it did not usually return to baseline for 48 hours following the initiation of the procedure. One patient required a 4-unit platelet transfusion for a platelet count of 17,000/mm^3.

No changes in hepatic or renal function could be attributed to this therapy. Identifiable toxic reactions were found only in the cardiovascular, pulmonary, and hematopoietic systems.

Tumor Response

In the eight procedures, four were associated with pain at the tumor site. However, no size decrease or change in these tumors could be documented.

DISCUSSION

Animal studies using ex vivo plasma perfusion over *S. aureus* have been very encouraging (5,6). In our animal studies, toxicity was minimal. As presented in this trial, man tolerates this therapy much differently than the dog, ie, with profound cardiorespiratory toxicity. This toxicity appeared to be related to the amount of the perfused plasma and the rate at which it was returned to the patients. That is, a reversal of the mean blood pressure fall began to occur within 3–8 minutes after the infusions were stopped, although complete reversal to baseline often did not occur for hours.

Although our animal trials were performed at a lower total dose of *S. aureus* (0.2 g/kg rather than 1 g/kg as in this trial), we do not believe this alteration plays a significant role in the observed toxicity. Our patients began to show cardiovascular and pulmonary responses with reinfusion of 100–300 ml of plasma exposed to *S. aureus*. Because of the technical problems of the filter chambers plugging, the total *S. aureus* (1 g/kg) was divided among several (two to 14) cartridges. This separation of the *S. aureus* now allows an examination of the role of the amount of active substance in the chambers and of this relationship to toxicity. We found that

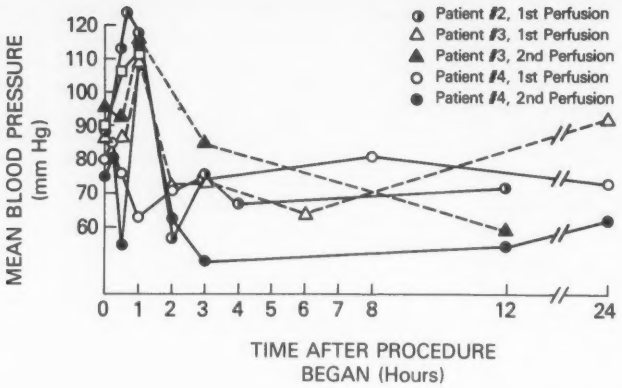

FIGURE 2.—Changes in the mean arterial blood pressure for each perfusion performed in the Critical Care Department, excluding the 5th patient who arrested during the 1st procedure.

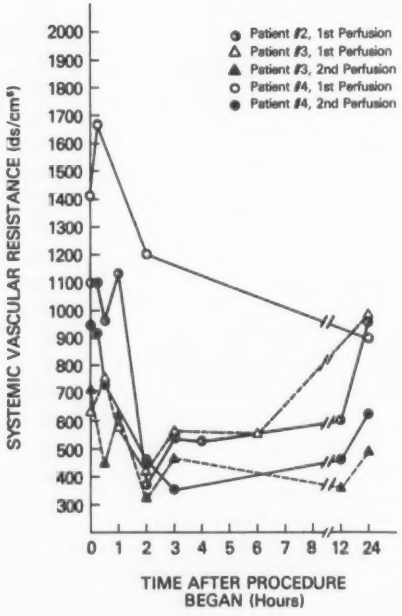

FIGURE 3.—Changes in systemic vascular resistance with time in 5 procedures performed in the Critical Care Department, excluding the 5th patient who arrested during the 1st procedure.

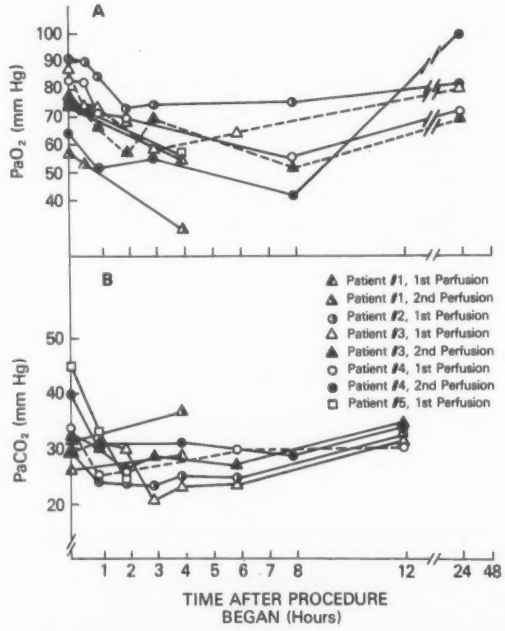

FIGURE 4.—A. Changes in PaO_2 in all patients treated with immunoperfusion with time. PaO_2 decreased in all procedures despite increases in respiratory rate. B. Changes in $PaCO_2$ in all patients treated with immunoperfusion. Decreases occurred in Patients 2–5 in conjunction with an increased respiratory rate.

the observable toxicity predictably began with 100–300 ml of infused plasma, which is the amount passed through the first cartridge only. The first cartridges contained between 0.5 g/kg (total, two cartridges) and 0.07 g/kg (total, 14 cartridges). Thus, the toxicity observed was present at levels well below those at which our dog trials were performed. It appears that toxicity correlates more closely with the amount of plasma exposed to the *S. aureus* and the rate reinfused, rather than the amount of *S. aureus* with which it comes into contact. These observations suggest that a product is generated or released in the ex vivo system that is related to the toxicity, and perhaps a slower rate of infusion would result in less toxicity. We are now examining the effluents in attempts to identify this product.

The animal responses have been very encouraging; however, the use of *S. aureus* as a source of protein A in the treatment of human malignancy must be approached with caution. It may be possible to avoid this toxicity by slowing the infusion rate of the exposed plasma. Trials using the native *S. aureus* in the future should examine this approach and carefully monitor cardiac, respiratory, and hematopoietic toxicity.

Our animal data suggest that nearly 100% of the plasma needs to be perfused and reinfused for response (6); however, the toxicity observed in our patient procedures inhibited our ability to reach this amount of plasma. This, perhaps, is one explanation of why objective tumor responses were not observed in this phase I trial. If this toxicity can be avoided, then it may be possible to perfuse larger volumes of plasma and this may translate into tumor response as in our animal studies. Another alternative is to use purified protein A rather than the whole *S. aureus* in a similar perfusion system. Terman et al (7) have reported tumor shrinkage with less toxicity using such a system.

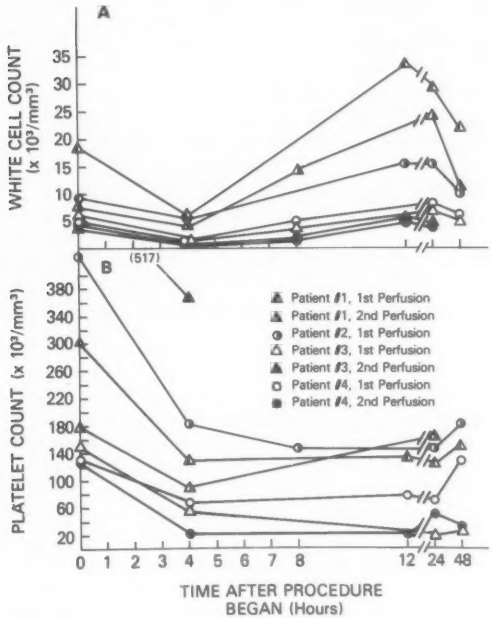

FIGURE 5.—A. Changes in total wbc count with time after initiation of *S. aureus* immunoperfusion. B. Changes in platelet count with time after initiation of *S. aureus*. 1 patient (No. 4) required a platelet transfusion to mantain a platelet count > 20,000/mm³.

REFERENCES

1. BALDWIN RW, PRICE MR, and ROBINS RA. Significance of serum factors modifying cellular immune responses to growing tumors. Br J Cancer 28:37, 1973.
2. KORNVALL G, SEAL US, FINSTAD S, ET AL. Phylogenetic insight into evolution of mammalian Fc fragment of G globulin using staphylococcal protein-A. J Immunol 104:140, 1970.
3. STEELE G, ANKERST J, and SJOGREN HO. Alterations in in-vitro antitumor activity of tumor-bearing serum by absorption with *Staphylococcus aureus* Cowan I. Int J Cancer 14:83, 1974.
4. BANSAL SC, BANSAL BR, THOMAS HC, ET AL. Ex-vivo removal of serum IgG in a patient with colon carcinoma: some biochemical, immunological and historical observations. Cancer 42:1, 1978.
5. TERMAN DS, YAMAMOTO T, MATTIOLI M, ET AL. Extensive neurosis of spontaneous canine mammary adenocarcinoma after extracorporal perfusion over *Staphylococcus aureus* Cowan I. J Immunol 124:795, 1980.
6. MESSERSCHMIDT GL, BOWLES C, ALSAKER R, ET AL. Long-term follow-up of dogs with spontaneous mammary tumors treated with ex-vivo plasma perfusion over *Staphylococcus aureus* Cowan I. Proc Am Assoc Cancer Res 23:279, 1982.
7. TERMAN DS, YOUNG JB, SHEARER WT, ET AL. Preliminary observations of the effects on breast adenocarcinoma of plasma perfused over immobilized protein-A. N Engl J Med 305:1195, 1981.

Doxorubicin-DNA Complex: A Phase I Clinical Trial[1,2]

Stephen W. Hall,[3,4,*] Robert S. Benjamin,[3] Michael A. Burgess,[3] Gerald P. Bodey,[3] Mario A. Luna,[3] and Emil J Freireich[3,5]

Thirty-five patients with advanced malignancies were treated with doxorubicin-DNA complex in a phase I clinical trial. The patients ranged in age from 30 to 77 years (median, 51) and received doxorubicin-DNA in doses that ranged from 20 to 135 mg/m^2. Of the doses administered, 73% were 60–90 mg/m^2 (median, 60). Nonhematologic toxic effects included nausea and vomiting in 32% of the patients, fever and chills in 24%, mucositis in 18%, and decreases in ECG QRS voltage of > 30% in 18%. Fever of unknown origin was seen in 26% of the patients, with documented infection in 9%; 6% had drug-related deaths. All patients experienced alopecia. Hematologic toxic effects (median day, Day 14) included median granulocyte count nadirs of 0.8 × 10^3/μl at a 60-mg/m^2 dose and 0.3 × 10^3/μl at 75 mg/m^2. Thrombocytopenia was less severe, with median nadir counts of 131,000 cells/μl at a 60-mg/m^2 dose on median day, Day 13. Treatment courses could be repeated every 3 weeks. Clinical responses (complete plus partial) were seen in 12% of the patients, with an additional 48% showing stable disease. No patients entered in the study were known to be doxorubicin-resistant. Based on these data, we recommend a starting dose of 60 mg/m^2 in patients with adequate bone marrow reserve and 50 mg/m^2 in those with compromised bone marrows. Doxorubicin-DNA exhibits major clinical toxic effects on the gastrointestinal and hematopoietic systems, with dose-limiting granulocytopenia. [Cancer Treat Rep 6:2033–2037, 1982]

Doxorubicin is one of the most active antitumor agents in man (1). It has a broad spectrum of anticancer activity; however, its maximum cumulative dose is limited by cardiotoxicity. The occurrence of doxorubicin cardiomyopathy currently limits the clinical use of the drug to approximately 550 mg/m^2 total dose, which generally occurs after 20–45 weeks of therapy (2). Despite intensive investigation of the cardiotoxicity of doxorubicin, no effective means has yet been devised that allows a significant increase in drug dose or duration of therapy without undue risk of cardiac toxicity.

In 1972, Trouet et al (3) proposed the concept of lysosomotropic chemotherapy. Their hypothesis was that a drug could be bound to a digestible carrier, taken up selectively only into cells with the capacity for endocytosis; be concentrated within lysosomes; and following digestion, be released within the cell. This approach to drug therapy would avoid drug distribution throughout the body fluids and tissues from passive diffusion. Drugs with cardiac toxicity seemed appropriate to study in this manner, since it was felt that cardiac muscle cells have low pinocytotic activity. Antitumor drugs were also attractive because some cancer cells have increased pinocytotic activity (4). The anthracycline antibiotics fit these criteria very well and, in addition, bind avidly to DNA. Theoretically, the anthracycline-DNA complex would be excluded from cardiac muscle cells and taken up by neoplastic cells. Once inside the

[1]Received Nov 2, 1981; revised Sept 17, 1982; accepted Sept 28, 1982.

[2]Supported in part by Public Health Service grant CA-05831 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and grants from Adria Laboratories, Wilmington, DE, and the Reno Cancer Center, NV.

[3]Departments of Developmental Therapeutics (*S. W. Hall, R. S. Benjamin, M. A. Burgess, G. P. Bodey,* and *E. J Freireich*) and Anatomical Pathology (*M. A. Luna*), the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]*Present address:* Department of Medicine, University of Nevada School of Medicine, Reno.

[5]We thank Dr. John Benvenuto, Ms. Judy Primeaux, and Ms. Charann Gamble for their assistance in the preparation of the drug.

**Reprint requests to:* Stephen W. Hall, MD, Department of Medicine, Division of Oncology/Hematology, Veterans Administration Medical Center, 1000 Locust St, Reno, NV 89520.

cells, the drug would be concentrated in the lysosomes where endonucleases would degrade the DNA, releasing the anthracycline to do its cellular damage.

Early experimental animal studies with doxorubicin-DNA complex suggest increased therapeutic ratios and decreased acute cardiac toxicity (5). Preliminary human clinical studies with anthracycline-DNA complexes have suggested patterns of toxicity and efficacy similar to those observed with the free drugs, with one notable exception, a lack of cardiac toxicity (6). This study is a report of our clinical phase I trial of doxorubicin-DNA complex.

MATERIALS AND METHODS

Drug Preparation

Doxorubicin-DNA complex was prepared precisely according to the method of Trouet et al (7) using herring sperm DNA dissolved in normal saline at a concentration of 2.34 g/L. The DNA solution was autoclaved within 24 hours of administration and doxorubicin was added (1 mg/5 ml of DNA-saline solution) just prior to administration. The drug solution is stable with ~ 1.5% degraded by plasma deoxyribonucleases each hour (8).

Phase I Study in Patients With Solid Tumors

Thirty-five patients, 15 males and 20 females ranging in age from 30 to 77 years (median, 51), with disseminated solid tumors, were entered in this study. The patients' clinical diagnoses are outlined in table 1. Five patients were previously untreated. The rest were refractory to one or more prior chemotherapeutic regimens, and 13 (37%) had received radiation therapy as well. Eleven patients (31%) had received prior doxorubicin; however, four of these had received only one or two doses in the weeks immediately preceding the doxorubicin-DNA complex administration and were considered to be untreated with doxorubicin in the analysis of response. No patient was known to be resistant to doxorubicin, since the patients who had previously received doxorubicin or doxorubicin-containing regimens had responded to the drug or drug regimen and subsequently relapsed when doxorubicin was discontinued. Informed consent was obtained from each patient

TABLE 1.—Doxorubicin-DNA phase I trial: clinical diagnosis and response

Diagnosis	No. of patients	No. of responses*				
		CR	PR	SD	PD	ED
Carcinoid tumor	4	1 (6)	1 (8)	1 (4)		1
Chondrosarcoma	1				1	
Leiomyosarcoma	2			1 (5)	1	
Nodular poorly differentiated lymphocytic lymphoma	2			2 (3,2+)		
Poorly differentiated malignant neoplasm	3			1 (3)	1	1
Carcinoma						
Gastric	3				3	
Thyroid	2			1 (4)	1	
Breast	3			1 (3+)	2	
Prostatic	1			1 (5+)		
Mediastinal choriocarcinoma (male)	1		1 (3)			
Renal	1			1 (3)		
Parotid acinar cell	2			1 (1)	1	
Hepatoma	1			1 (3)		
Lung						
Adenocarcinoma	5		1 (4)	1 (3+)	2	1
Small cell	1					1
Squamous cell	1			1 (7)		
Salivary gland adenoid	1			1 (3)		
Pancreas	1			1 (5)		
Total	35	1	3	15	12	4
% response in patients entered		3	9	43	34	11
% response in evaluable patients		3	9	48	39	
		12				
		60				

*Values in parentheses = response durations in mos. SD = stable disease, PD = progressive disease, ED = early death.

according to institutional policies, prior to initiation of the study.

The drug was administered at a rate of approximately 100 ml of doxorubicin-DNA complex solution per hour, necessitating a 4–6-hour infusion for an average dose. More rapid infusion resulted in severe chills and fever. Extreme care was taken to avoid extravasation. The drug was given iv at 3–4-week intervals, depending on myelosuppression. The dose ranged from 20 to 135 mg/m^2 (median, 60) with 73% of the doses between 60 and 90 mg/m^2. Starting doses of doxorubicin-DNA were based on the known toxic effects of free doxorubicin, and too few patients received doxorubicin-DNA doses < 60 or > 95 mg/m^2 to make analysis of these doses meaningful. The initial dose was generally 60–75 mg/m^2 depending on marrow reserve, with appropriate dose modifications made for abnormalities of liver function according to those routinely employed for doxorubicin alone (9), and subsequent doses were escalated or reduced depending on toxicity from the previous course. All drug doses in milligrams per square meter refer to doxorubicin concentration.

Complete response (CR) was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks, together with the disappearance of all tumor-related symptoms. Partial remission (PR) was defined as a 50% decrease in the sum of the products of the largest perpendicular diameters of all measured lesions lasting at least 4 weeks, in the absence of significant increase in any one lesion or the appearance of new lesions. Stable disease included tumor regression less than a PR lasting a minimum of 4 weeks or a steady state of response less than progressive disease of at least 8 weeks' duration. Progressive disease was defined as an unequivocal increase of any measured lesion by 25% or the appearance of a new lesion or lesions. Cardiotoxicity was diagnosed clinically by congestive heart failure without another established etiology; electrocardiographically by a $> 30\%$ decrease in the sum of the QRS voltage in the six limb leads as described by Minow et al (10); and in selected patients by endomyocardial biopsy.

RESULTS

Toxicity

Nonhematologic toxicity was evaluable in 114 courses given to 35 patients (table 2). As mentioned previously, doses of doxorubicin-DNA complex ranged from 20 to 115 mg/m^2 depending on marrow reserve and liver function. Fever and chills was a frequently mentioned side effect and was most prominent when the drug was administered rapidly. Slowing of the infusion rate to $\leq$ 100 ml/hour and premedication with antihistamines abolished or reduced these side effects so that subsequent doses could be given without their recurrence. Nausea and vomiting was noted in 11 patients and, although not reported, may have occurred in more. Nausea and vomiting cleared rapidly within 24 hours in most patients and was never dose-limiting. Alopecia was universal in patients receiving multiple courses of the drug. Mucositis was seen in six patients; it was severe in two. There were nine episodes of fever, with infection documented in three. The infections included two episodes of pneumonia and one of septicemia. There were nine episodes of fever of unknown origin, which resolved during antibiotic therapy. All episodes of fever and infection occurred while patients were neutropenic ($<$ 1000/mm^3 absolute granulocytes). There were two possible drug-related deaths: one from progressive pneumonia that was unresponsive to antibiotic and one due to an acute pulmonary hemorrhage in a patient with a normal platelet count. The latter patient had presump-

TABLE 2.—Nonhematologic toxicity of doxorubicin-DNA in 35 patients*

Toxic effect	No. of episodes in 114 courses (%)	No. of patients† (%)
Nausea and vomiting	15 (13)	11 (32)
Hives	2 (2)	2 (6)
Fever and chills (related to drug administration)	8 (7)	8 (24)
Alopecia	—	(100)
Cardiac toxicity ($> 30\%$ decrease in QRS voltage)	—	6 (18)
Mucositis	6 (5)	6 (18)
Diarrhea	1 (1)	1 (3)
Extravasation	2 (2)	2 (6)
Documented infection	2 (2)	3 (9)
Fever of unknown origin	9 (8)	9 (26)
Drug-related death	—	2 (6)

*Dose range, 20–135 mg/m^2 (median, 60).
†Alopecia was universal in patients receiving multiple courses of therapy.

tive evidence of tumor involvement in the mediastinum and died unexpectedly 7 days after drug administration. Tumor necrosis or progression may have caused this fatal hemorrhage. No evidence of hepatic or renal drug toxicity was encountered in the study. Because of the paucity of patients treated at variable doses, no definite relationship between dose and nonhematologic toxicity can be determined.

Hematologic toxicity in this phase I study could only be evaluated at three dose levels: 60, 75, and 90 mg/m^2 (table 3). There were too few courses at other doses to make an analysis possible. Patients treated at these doses had grossly normal liver function tests and blood cell counts recorded at least weekly and frequently twice weekly. The major myelotoxic effects were leukopenia and granulocytopenia. The median nadirs were seen on Day 14; the median wbc count nadirs were 2300 and 1500/μl and the median granulocyte count nadirs were 800 and 320/μl for the 60- and 75-mg/m^2 doses, respectively. The median platelet count nadir occurred on Days 12 and 13, with counts of 131,000 and 155,000/μl for the 60- and 75-mg/m^2 doses. Granulocyte recovery was complete and a second cycle of chemotherapy was administered 23–24 days (median) after the first course. There was no evidence of cumulative myelosuppression with repeated courses of therapy.

Cardiac abnormalities were seen in two patients. The first patient had received prior doxorubicin, cyclophosphamide, and chest radiotherapy before doxorubicin-DNA therapy. After a dose of 150 mg/m^2 the ECG voltage decreased about 30%. The patient died shortly after this from infectious complications. At autopsy, there was evidence of arteriosclerotic cardiovascular disease but no evidence of doxorubicin cardiomyopathy. The second patient was elderly and had pre-existing heart disease; he had not received prior doxorubicin chemotherapy. However, after 290 mg/m^2 of doxorubicin-DNA complex, he developed a 35% decrease in QRS voltage. The patient was known to have pre-existing heart disease and cardiac enlargement, which had not changed. Cardiac function studies were attempted without success, and he was treated with digoxin. The patient's neoplasm had just begun to progress at that time and the drug was discontinued.

Response

Of the 35 patients entered in the study, four died within 3 weeks of entry from progressive tumor or complications related to their disease or chemotherapy. There were 31 patients, therefore, who were considered to be evaluable for tumor response (table 1). In these patients, there was one CR (3%) lasting 6+ months, and there were three PRs (9%) lasting 8, 4, and 3 months. Fifteen patients (48%) had stable disease. Five of those patients (16%) showed tumor regression less than PR lasting 7+, 4, 3+, and 2 months. The other ten patients had disease stabilization for a median of 3 months (range, 2–5+). Twelve patients (39%) had progressive disease. Two patients with carcinoid tumors responded to doxorubicin-DNA (one CR, one PR), as did a male patient with choriocarcinoma (PR) and a female with lung adenocarcinoma (PR). Disease stabilization or improvement was noted in other doxorubicin-responsive tumors (table 1). None of the responders (CR + PR) and only one of the patients with tumor regression less than PR had received prior doxorubicin. This patient was known to have doxorubicin-sensitive disease and was given doxorubicin-DNA after 500 mg/m^2 of doxorubicin, with continued improvement of her neoplastic process.

DISCUSSION

The response rate of 12% (CR + PR) in the phase I study is less than expected from patients who had received no prior treatment with doxorubicin. However, 12 of our patients had received prior doxorubicin, and a majority had received multiple prior chemotherapeutic regimens (median, three). Sixty-three percent of the patients showed tumor regression or disease stabilization, suggesting definite drug activity. Two of the four responses occurred in patients with carcinoid tumor, a malignancy recently reported to be responsive to doxorubicin therapy (11). There was a paucity of

TABLE 3.—Doxorubicin-DNA: hematologic toxicity*

Dose (mg/m^2)	No. of courses	Wbc count nadir, × 10^3 cells/μl	Granulocyte count nadir, × 10^3 cells/μl	Day of nadir	Platelet count nadir, × 10^3 cells/μl	Day of nadir	Day of next course of therapy†
60	35				131 (7–410)	13 (8–19)	23 (19–33)
	37	2.3 (0.6–4.7)	0.80 (0–2.6)	14 (4–23)			23 (19–33)
75	20	1.5 (0.6–13.1)	0.32 (0–10.0)	14 (11–20)	155 (16–384)	12 (8–16)	24 (14–34)
90	7	2.4 (0.1–4.0)	1.10 (0–21.0)	14 (7–20)	111 (66–292)	9 (6–15)	21 (19–30)

*Values are medians, with ranges in parentheses.
†Granulocyte count > 1.5 × 10^3 cells/μl; platelet count > 100 × 10^3 cells/μl.

patients in this study, previously treated or untreated, with more common tumors that are doxorubicin-responsive (lymphomas, sarcomas, small cell bronchogenic carcinoma, and carcinoma of the breast), since they receive doxorubicin in primary combination therapy in our department. Thus, an accurate comparison of the response rates in this study with those expected for free doxorubicin is not possible.

Because of prior studies on the acute toxic effects of doxorubicin (12), we began our phase I study of doxorubicin-DNA at comparable doses. This allowed us to rapidly determine the maximum tolerated dose in a small number of patients. Dose-limiting toxicity was myelosuppression, particularly granulocytopenia, subjecting patients to increased risk from infection. Considering that 84% of the patients had received extensive prior chemotherapy and that 37% had received prior radiation therapy, the leukopenia seen was similar to that for doxorubicin given by intermittent infusion at comparable doses.

The incidence of mucositis in our study (18% of the patients) was slightly higher than that seen with intermittent, high-dose, single-dose doxorubicin (9). Two episodes of severe mucositis were seen in one patient who had abnormalities of liver function tests and one who was given a dose of 90 mg/m^2. In a preliminary phase I study with doxorubicin-DNA complex (75 mg/m^2) in 12 patients, Rozencweig et al (13) found a slightly greater incidence (30%) of mucositis. The incidence and severity of most other acute toxic effects in our study were similar to those found by Rozencweig et al and those seen with free doxorubicin.

The incidence of hives and fever and chills was significant in the early studies and appeared to be clinically similar to a histamine-release phenomenon. This was definitely related to rapid drug infusion and was perhaps a reaction to the DNA. The reaction was largely eliminated by slowing the rate of infusion to < 100 ml/hour and premedicating the patients with antihistamines. Despite the inconvenience of prolonged infusions and the increased risk of extravasation that accompanies it, we cannot recommend more rapid drug administration.

The major hope of the lysosomotropic drug is to eliminate or reduce cardiac toxicity attributed to doxorubicin. Unfortunately, our patients did not receive enough cumulative doses of the drug to allow us to determine if there is decreased doxorubicin cardiotoxicity when the drug is bound to DNA. This was true of the patients of Rozencweig et al as well.

In this study, we were able to determine that a dose of 50–60 mg/m^2 of doxorubicin-DNA every 3 weeks had tolerable toxicity similar to that of doxorubicin.

Based on the results of these studies, we conclude that doxorubicin-DNA has significant antitumor activity and we currently recommend a starting dose of 50 mg/m^2 in patients with compromised bone marrow reserve and a starting dose of 60 mg/m^2 in patients with adequate bone marrow reserve and normal liver function. The drug can be repeated at 3–4-week intervals depending on myelotoxicity and may be escalated approximately 25% if there is no evidence of drug toxicity. Appropriate dose modifications should be made for liver dysfunction or when the drug is used with other myelosuppressive drugs. The major clinical toxic effects of doxorubicin-DNA are gastrointestinal and hematopoietic, with dose-limiting granulocytopenia.

REFERENCES

1. O'Bryan RM, Luce JK, Talley RW, et al. Phase II evaluation of Adriamycin in human neoplasia. Cancer 32:1–8, 1973.
2. Lefrak EA, Pitha J, Rosenheim S, et al. A clinicopathologic analysis of Adriamycin cardiotoxicity. Cancer 32:302–314, 1972.
3. Trouet A, Deprez-de Campeneere D, and De Duve C. Chemotherapy through lysosomes with a DNA-daunorubicin complex. Nature 239:110–112, 1972.
4. Sutton CH, and Becker NH. Lysosomes and pinocytosis in human and experimental brain tumors. Ann NY Acad Sci 159:497–508, 1969.
5. Langlet A, Pye I, and Lie SO. Decreased cardiac toxicity of Adriamycin and daunorubicin when bound to DNA. Acta Pharmacol Toxicol 35:379–385, 1974.
6. Michaux JL, Cornu G, and Trouet A. Preliminary clinical trials with the Adriamycin-DNA complex in human leukemias and non-Hodgkin's lymphomas. *In* Adriamycin Review, Part IV. Ghent, Belgium, European Press Medikon, 1975, pp 215–225.
7. Trouet A, Deprez-de Campeneere D, De Smeldt-Malengreu M, et al. Experimental leukemia chemotherapy with a "lysosomotrophic" Adriamycin-DNA complex. Eur J Cancer 10:405–411, 1974.
8. Trouet A, Deprez-de Campeneere D, Zenebergh A, et al. Lysosomotropic cancer chemotherapy with Adriamycin-DNA. *In* Adriamycin Review, Part I. Ghent, Belgium, European Press Medikon, 1975, pp 62–69.
9. Benjamin RS. A practical approach to Adriamycin (NSC-123127) toxicology. Cancer Chemother Rep Part 3, vol 6(2):191–194, 1975.
10. Minow RA, Benjamin RS, and Gottlieb JA. Adriamycin (NSC-123127) cardiomyopathy—an overview with risk factors. Cancer Chemother Rep Part 3, vol 6(2):195–201, 1975.
11. Soloman A, Sonoda T, and Patterson FK. Response of metabolic malignant carcinoid tumor to adriamycin (NSC-123127). Cancer Treat Rep 60:273–275, 1976.
12. Benjamin RS, Wiernik PH, and Bachur NR. Adriamycin chemotherapy—efficacy, safety and pharmacologic basis of an intermittent high dose schedule. Cancer 33:19–27, 1974.
13. Rozencweig M, Kenis Y, Attassi G, et al. DNA-adriamycin complex: preliminary results in animals and man. Cancer Chemother Rep Part 3, vol 6(2):131–136, 1975.

Phase I Study of SOAz[1,2]

S. Nasca, D. Jezekova, P. Coninx,* E. Garbe, Y. Carpentier, and A. Cattan[3,4]

SOAz, an inorganic cyclic derivative, was given as a single iv injection through one cycle in a phase I trial. Dose-limiting toxic effects noted were neutropenia and thrombocytopenia occurring at doses ≥ 220 mg/m² in patients with prior myelosuppressive treatment. The time to nadir for blood cell counts was long, as well as the time to recovery, so cumulative toxicity could be suspected. No other significant toxicity was noted. In patients with measurable tumor volume, no significant antitumor responses were seen. For further study, the dose of 220 mg/m² every 6–8 weeks according to hematologic restoration is recommended. [Cancer Treat Rep 66:2039–2042, 1982]

SOAz (1), an inorganic cyclic derivative, is a relative of MYKO 63 (hexaziridinocyclotriphosphazene) (2). This latter drug was successfully tested on several experimental tumors (3), but its therapeutic index was disappointing because of cumulative toxicity. Chemists therefore studied other molecules and synthesized SOAz (pentaziridinocyclodiphosphathiazene; fig 1) (4,5). Animal toxicity trials of this drug were conducted in mice, rats, beagle dogs, and monkeys.[5] The most prominent finding was hematologic toxicity with leukopenia and thrombocytopenia but without cumulative effect. The activity of SOAz was described in vitro against various tumors of human or animal origin including S lymphoma, R lymphoma, lymphosarcoma, neuroblastoma, rhabdomyosarcoma, fibroblastoma, ovarian carcinoma, HeLa cervix carcinoma, Ehrlich ascites carcinoma, osteosarcoma, subcutaneous melanoma, and Lewis lung carcinoma (6). Activity of SOAz was reported against several rodent tumors in vivo, including P388 and L1210 leukemias, Lewis lung carcinoma, subcutaneous B16 melanoma, Sarcoma 180, and Y. M. 12 sarcoma (4,7).[5] Furthermore, encouraging therapeutic results were obtained in spontaneous osteogenic sarcoma of the dog without any hematologic toxicity. Although the mechanism of action of SOAz was unknown, this drug was selected for a phase I study on the basis of a possible high therapeutic index. The trial was designed to determine the maximum tolerated dose (MTD) for a single iv injection.

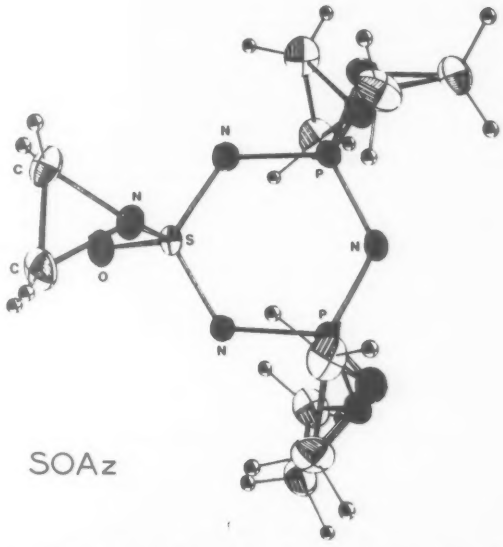

FIGURE 1.—Compound structure with conventional chemical symbols.

[1]Received May 17, 1982; revised Sept 14, 1982; accepted Sept 28, 1982.

[2]Supported in part by grants from CNRS-PIRMED and ARERS.

[3]Institut Jean Godinot, Reims, France.

[4]We thank H. Roelandt and C. Galdies for assisting in this study.

[5]Unpublished results from Otsuka Chemical Co, Ltd, Osaka, Japan.

**Reprint requests to:* P. Coninx, MD, Department of Medicine, Institut Jean Godinot, B. P. 171, 1, Avenue Général Koenig, 51056 Reims Cedex, France.

PATIENT SELECTION

Patients with a histologically confirmed diagnosis of malignancy were eligible for this study regardless of age, tumor site, and previous therapy, provided that the tumors were refractory to conventional modalities of treatment or that no effective therapy was known. Informed consent from all patients was obtained and none were given radiotherapy or chemotherapy within the 4 weeks prior to SOAz administration. Criteria for entry in this study included a performance status ≥ 40 (Karnofsky scale) (8), an expected survival time of at least 8 weeks, an adequate hematologic status (granulocyte count ≥ 2000/μl and platelet count ≥ 100,000/μl), an acceptable renal creatinine (≤ 1.7 mg/100 ml), and acceptable hepatic function (bilirubin and transaminases < 1.5 times normal value).

TREATMENT SCHEDULE

SOAz was supplied as a dry powder without excipients by the Otsuka Chemical Co, Ltd. SOAz vials (300 mg) were reconstituted in 5 ml of sterile normal saline. All doses were administered as a 5-minute rapid infusion through a free-flowing iv line. The starting dose was 65 mg/m^2, based on $^1/_3$ of the minimal toxic dose in the most sensitive animal species (dog), and dose escalation (table 1) was carried out according to Fibonacci's search scheme (9). Each patient was given a single dose through one cycle; at least three patients were treated at each dose level and were observed until recovery occurred before other patients were entered at a higher dose level. As soon as the MTD was determined, other patients were entered at lower dose levels (six patients) or at the MTD level (12 patients) (table 1) so that the toxic manifestation could be studied accurately.

STUDY PARAMETERS

Pretreatment assessment included a complete history and physical examination with documentation of metastatic disease and objective tumor measurement when possible. Laboratory study included a cbc with differential and platelet count, chemistry panel (BUN, creatinine, electrolytes, glucose, total protein, albumin, total bilirubin, SGOT, SGPT, alkaline phosphatase, and fibrinogen), urinalysis, ECG, and chest roentgenogram. Follow-up included weekly cbc with differential and platelet count; chemistry panel, urinalysis, and ECG were checked at 1- and 3-week intervals. Repeated physical examinations with tumor measurement were performed at 3-week intervals. All the investigations included in patient assessment were carried out again at the time when recovery from toxic effects occurred.

When we became aware of the long period for hematologic recovery, we elected to study eight patients, the last to be entered in the trial, to determine the blood cell kinetics with higher accuracy at MTD. Therefore, a weekly follow-up through a period of 8 weeks was carried out. Toxicity was defined by standard World Health Organization (WHO) criteria (10).

TABLE 1.—Relationship between dose and No. of patients

Dose (mg/m^2)	No. of patients	
	Total	Men/women
65	6	3/3
133	6	3/3
220	12	8/4
330	3	3/0
467	2	2/0

RESULTS

Patient characteristics are detailed in table 2. One of the patients, who had been erroneously selected for this study, could not be evaluated because he died from progressive disease soon after SOAz administration. The number of individuals treated at each dose level is

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Total	30
Evaluable	29
Sex	
Men	19
Women	10
Median age in yrs (range): 69 (27–81)	
Previous treatment	
Chemotherapy only	5
Radiotherapy only	10
Chemotherapy and radiotherapy	12
Disease	
Squamous cell carcinoma	
Head and neck	8
Cervix	3
Anus	1
Esophagus	1
Bronchus	1
Basal cell carcinoma	
Skin	1
Adenocarcinoma	
Colorectum	4
Prostate	2
Stomach	1
Kidney	1
Breast	1
Endometrium	1
Unknown	2
Sarcoma	
Soft tissue	1
Osteosarcoma	1

TABLE 3.—Myelosuppressive toxicity: median nadir blood cell count and median day of nadir

Dose (mg/m²)	Polymorphonuclear cells		Mononuclear cells		Platelets	
	Median count* (range)	Median day (range)	Median count* (range)	Median day (range)	Median count* (range)	Median day (range)
65	3.1 (2.5–5.2)	21 (8–22)	0.5 (0.4–1.1)	19 (7–25)	163 (61–207)	22 (14–31)
133	3.1 (0.8–4.5)	19 (7–35)	0.5 (0.1–1.4)	17 (7–35)	158 (22–218)	24 (15–43)
220	1.7 (0.3–4.0)	30 (15–43)	0.5 (0.1–0.8)	28 (18–36)	74 (11–195)	28 (18–36)
330	0.6 (0.4–1.1)	28 (27–29)	0.1 (0.1–0.2)	29 (21–35)	48 (32–55)	23 (21–28)
467	0.1 (0.1–0.2)	25 (22–27)	0.1 (0.1–0.1)	25 (22–27)	31 (25–37)	21 (21–22)

* $\times 10^3$ cells/μl.

shown in table 1. No patient receiving SOAz showed an unacceptable hepatic function, whereas the creatinine levels were high in four patients: a dose of 65 mg/m² (creatinine, 1.5 and 1.6 mg/100 ml), two patients; 133 mg/m² (creatinine, 1.5 mg/100 ml), one; and 220 mg/m² (creatinine, 1.7 mg/100 ml), one. Hematologic toxicity was severe in some cases but never lethal. For the four patients with a high creatinine level, the bone marrow depletion did not seem to be more severe. As shown in table 3, neutropenia and thrombocytopenia were dose-related and became dose-limiting at 220–330 mg/m². Platelet variations after SOAz administration at a dose of 330 mg/m² are documented in figure 2. The same pattern was observed for polymorphonuclear cells, with cell count nadir at Week 4 and recovery at Weeks 6–7. Blood transfusions were required for six patients who were given SOAz at a dose level > 65 mg/m²: one at 133 mg/m², four at 220 mg/m², and one at 467 mg/m². For these patients the median fall in hemoglobin was 4.6 g/100 ml (range, 3.1–6.5) versus 2.3 g/100 ml (range, 0.31–3.7) for the others who did not receive blood transfusions. The median day of the erythrocyte count nadir was Day 35 (range, 15–56).

In the eight patients who were given SOAz at a dose of 220 mg/m² and who had had weekly wbc counts for 8 weeks, the median recovery times were 50 days (range, 38–60) for polymorphonuclear cells and 46 days (range, 38–50) for platelets. Of these patients, four had to be given blood transfusions because their hemoglobin was < 7.5 g/100 ml. Furthermore, it appeared that the more extensive prior myelosuppressive treatment was, the deeper the nadir cell counts, the sooner their occur-

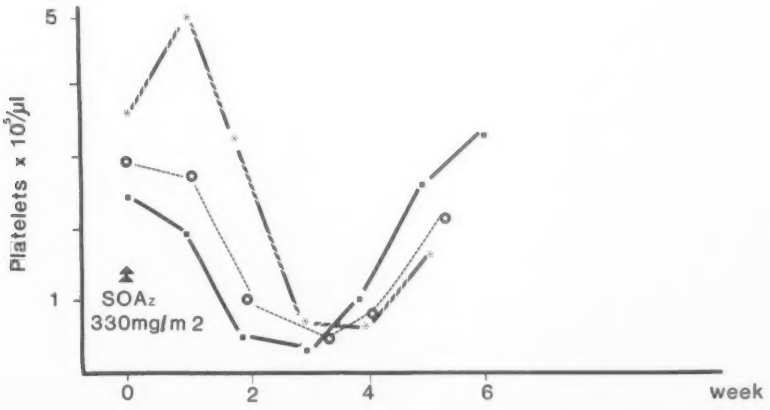

FIGURE 2.—Platelet counts after iv injection of 330 mg/m² of SOAz in 3 patients.

rences, and the later the recoveries.

Other toxic effects may be considered as negligible since they occurred infrequently and were of low grade (1 or 2) according to WHO criteria. Fever was observed in six patients, nausea and vomiting in two, and diarrhea in one. Immediately after infusion, two male patients also reported a moderately painful, strangling sensation in the chest without any symptoms or signs of heart disease. Another patient reported a fleeting sensation of mint breath during infusion.

The antitumor effect could be assessed in 24 patients. There were no complete or partial responders according to WHO criteria (10). Six patients achieved disease stabilization and 18 showed tumor progression.

DISCUSSION

The MTD of SOAz as a single dose is 220 mg/m^2 for patients with extensive prior myelosuppressive treatment. Neutropenia and thrombocytopenia, which were the main toxic effects, occurred almost simultaneously and recovery times were similar. These hematologic toxic effects were delayed, so the treatment could be repeated only about 40–60 days after the first administration. Another trial is in progress to determine a possible cumulative effect. Other manifestations of drug toxicity were negligible.

Although SOAz did not seem to be effective in this phase I study, the lack of antitumor activity should not preclude the initiation of phase II trials with this compound, but only after cumulative toxicity has been studied. The assessment of SOAz activity was limited in this study by the structure of the phase I trial; patients with extensive prior chemotherapy or radiotherapy were selected and low doses of SOAz were required. For further trials with this compound given as a single iv injection, we suggest the dose of 220 mg/m^2 every 6–8 weeks (according to hematologic restoration) for patients with prior myelosuppressive treatment, and we cautiously propose 330 mg/m^2 every 6–8 weeks for the patients who are not given such prior therapy.

REFERENCES

1. Galy J, Enjalbert R, Van Der Huizen AA, et al. Structures of inorganic rings as antitumor agents. I. Structure of the two allotropic varieties of SOAz, $(NPAz_2)_2(NSOAz)$. Acta Cryst B37:2205, 1981.
2. Galy J, Enjalbert R, and Labarre JF. The first example of a cyclophosphazene "anticlathrate structure": geometry of MYKO 63 from an X-ray investigation of the complex MYKO 63-3CCl_4. Acta Cryst B36:392, 1980.
3. Labarre JF, Faucher JP, Levy G, et al. Antitumor activity of some cyclophosphazenes. Eur J Cancer 15:637, 1979.
4. Labarre JF, Sournies F, Van De Grampel JC, et al. Nouveaux composés à cycle inorganique utiles comme médicament et procédé pour leur préparation. French ANVAR Patent No. 79-17336, July 4, 1979 (world extension: July 4, 1980).
5. Van De Grampel JC, Van Der Huizen AA, Jekel AP, et al. Derivatives of *cis*-$NPCl_2$ $(NSOCl)_2$ and $(NPCl_2)_2NSOCl$. XVI. The preparation of some aziridino derivatives of $(NPCl_2)_2NSOX$ (X = F,Az,Ph) with a potential anticancer activity. Inorg Chim Acta 53:L169, 1981.
6. Van Der Huizen AA, Jekel AP, Van De Grampel JC, et al. *In* Proceedings of the 3rd International Symposium on Inorganic Ring Systems (IRIS Meeting), GRAZ, Aug 1981, p 34.
7. Labarre JF, Sournies F, Cros S, et al. New designs in inorganic ring systems as anticancer drugs. Antitumor activity of the aziridino derivatives $(NPAz_2)_2NSOX$ with X = F,Az,Ph. Cancer Lett 12:245, 1981.
8. Karnofsky DA. Chemotherapy of the lymphoma. *In* Proceedings of the International Conference of Leukemia-Lymphoma. Philadelphia, Lea and Febiger, 1968, vol 1, pp 409–422.
9. Carter SK, and Goldsmith M. The development and clinical testing of new anticancer drugs at the National Cancer Institute. Recent Results Cancer Res. *In* Platinum Coordination Complexes in Cancer Chemotherapy (Connors T, and Roberts J, eds). New York, Springer-Verlag, 1974, pp 138–144.
10. WHO Handbook for reporting results of cancer treatment. Geneva, WHO, 1979.

Medroxyprogesterone Acetate (MAP) Relative Bioavailability After Single High-Dose Administration in Cancer Patients[1]

Franco Pannuti,[2,*] Carlo M. Camaggi,[3] Elena Strocchi,[4] Michele Giovannini,[2] Anna R. Di Marco,[4] and Barbara Costanti[4]

A pharmacokinetic evaluation of medroxyprogesterone acetate (MAP; 6α-methyl-17α-hydroxyprogesterone acetate) was undertaken in 70 patients with advanced cancer treated either orally or im with single high doses. MAP plasma levels were determined by gas chromatography (^{63}Ni electron capture detector) after derivatization with heptafluorobutyric anhydride, using 17α-hydroxyprogesterone caproate as internal standard. Plasma levels after oral administration can be approximated by a triexponential function in agreement with a pharmacokinetic two-compartment open model with first-order absorption; peak levels are dose-related, but high interindividual variance is present. Following im administration, both decay phases are masked by prolonged absorption; peak levels are lower than after oral administration, but long-term bioavailability is higher, as demonstrated by comparison of the values for areas under curves in the 0–146-hour interval. The high interindividual ranges in the observed MAP plasma levels indicate that if any significant clinical evaluation is to be made, routine analysis of plasma MAP is mandatory. [Cancer Treat Rep 66:2043–2049, 1982]

Medroxyprogesterone acetate (MAP; 6α-methyl-17α-hydroxyprogesterone acetate) is a well-known synthetic progestogen whose action against hormone-dependent tumors has been recognized since the early 1960s. Only recently, however, extensive clinical trials have clearly pointed out that its effectiveness in advanced breast cancer therapy is greatly enhanced if it is used in high daily doses (500–2000 mg) (1–6). This behavior parallels the findings of in vitro studies, showing that MAP nearly inhibits the growth of the MCF-7 human breast cancer tumor cell line at 1.10^{-6} M concentration, whereas it is ineffective at the lower 1.10^{-8} M level (7). In addition, high-dose MAP (≥ 500 mg/day) was found to be useful in the therapy of renal, prostatic, and endometrial tumors as well as in oncologic pain control (8–10).

After either oral or im administration, MAP is absorbed but no extensive bioavailability studies on this drug at different doses are reported. We now present the results of our study in which plasma levels of MAP were determined by gas chromatography in 70 cancer patients after oral or im administration of a single high dose.

MATERIALS AND METHODS

Chemicals (highest analytic grade) were supplied by E. Merck (Darmastadt). MAP (Farmitalia, Milan) and 17α-hydroxyprogesterone caproate (Shering; internal standard) were recrystalized several times until chromatographic purity. Chromatographic analyses were carried out on a Varian 3700 automatic gas chromatograph equipped with a ^{63}Ni electron capture detector, autosampler model 8000, and CDS 111 data system.

Statistical analysis of the results was accomplished with the SPSS program package (11). Nonlinear least-squares fit of the plasma decay of MAP concentrations was obtained using the Gauss-Newton algorithm as programmed in the BMDP package (12).

Patients entered in this study had histologically proved advanced cancer and were assessed as having normal liver and renal functions by standard clinical tests. All

[1]Received Aug 5, 1981; revised Sept 7, 1982; accepted Sept 21, 1982.
[2]Divisione di Oncologia, Ospedale M. Malpighi, Bologna, Italy.
[3]Istituto di Chimica Organica, Università degli Studi Viale Risorgimento, Bologna, Italy.
[4]Associazione Nazionale per lo Studio e la Cura dei Tumori Solidi, Divisione di Oncologia, Ospedale M. Malpighi, Bologna, Italy.
Reprint requests to: Prof. Franco Pannuti, Divisione di Oncologia, Ospedale M. Malpighi, 15 Bologna, Italy.

had received prior chemotherapy or radiation therapy, but oncologic treatments were discontinued at least 1 month before (and any other treatment at least 2 days before) the time of this study. Life expectancy was ⩾ 2 months.

Each patient received a single dose of MAP either orally (500, 1000, 2000, or 3000 mg) or im (500, 1000, or 2000 mg) (Farlutal depot vials; ten patients for each treatment). Oral doses were administered in fruit juice. Blood samples were collected before and at various times after MAP administration (1, 2, 4, 6, 8, 10, 26, 30, 50, 74, 98, and 146 hours) up to the seventh day. In all experiments venous blood samples were taken in heparinized tubes and centrifuged. Plasma samples were kept frozen at –20°C until analysis.

Extraction and Derivatization Procedure

As previously proposed by Kaiser and co-workers (13), MAP was analyzed after derivatization with heptafluorobutyric anhydride as 3-enol heptafluorobutyrate. A 1-ml plasma sample, 100 µl of a stock solution (containing about 200 ng of 17α-hydroxyprogesterone caproate as internal standard), and 1 ml of distilled water were combined in an 18 × 100-mm silanized glass centrifuge tube. This mixture was extracted twice in a horizontal shaker with 3 + 3 ml of cyclohexane. The organic phase was then completely evaporated under nitrogen at 30°C, leaving a residue which was then directly treated with 1 ml of acetone:acetonitrile (1:1) plus 50 µl of heptafluorobutyric anhydride. After 1 hour at 30°C, this solution was evaporated under nitrogen without further heating. The residue was reconstituted with 1 ml of cyclohexane in a vortex mixer and transferred in the standard minivial of the chromatograph's autosampler programmed for 1-µl injections.

Chromatographic Analysis

Several stationary phases and conditioning of the chromatographic column were tested in trial runs. Considerable separation of the 3-enol ester of MAP and internal standard from the other components of the injected mixture was achieved with several commercially available OV-1 or OV-17 silicones on silanized Chromosorb W glass columns; but an accurate reproduction of the analytic results was obtained only after using 3% Supelco SP-2250 on 100–120 Supelcoport. The analytic method was calibrated using blank plasma samples (1 ml) spiked with various amounts of MAP and 17α-hydroxyprogesterone caproate (internal standard). The typical run-to-run coefficient of variation was < 8% for plasma levels > 2 ng/ml. No interferences from endogenous or exogenous materials were observed; however, since MAP is a highly stable and insoluble compound, extreme care is necessary in cleaning all the glassware (chromic acid), and plasma samples must be collected with disposable syringes to avoid contamination problems.

The absolute minimal detectable quantity of MAP is about 0.5 pg per chromatographic injection; however, the accuracy of the method decreases for plasma levels < 2 ng/ml, because of cleanup problems. The detector response is linear in the 1–500-pg range (corresponding to plasma levels of 1–500 ng/ml).

All of the routine analyses were carried out with the following chromatographic conditions: 2 m × 3 mm (inside diameter) silanized column (3% SP-2250 on 100–120 Supelcoport, preconditioned for 1 week at 340°C); injection port temperature, 270°C; detector temperature, 300°C; column oven temperature program, 230°C to 270°C at 2°C/minute; carrier gas (nitrogen), 30 ml/minute; and injections of 1 µl. A blank plasma sample was included with every batch processed. Calibration runs were carried out weekly without detection of noticeable changes in the relative response factor.

A typical gas chromatographic analysis is shown in figure 1.

RESULTS

Mean plasma levels of MAP following single-dose administration are reported in semilog plots in figures 2 and 3, where each value is the mean of results from the ten patients receiving each dose. The individual time/concentration profile of each patient shows some discontinuities, but can be interpolated by a triexponential function (14).

$$C = -A.e^{\alpha t} + B.e^{\beta t} + C.e^{\gamma t}.$$

The half-life parameters obtained by the best-fit procedure are reported in table 1. After oral administration, plasma levels rapidly increase, reaching a peak after 1–2 hours (fig 2), followed by a biexponential decay. The decaying process involves an initial half-life of about 1.4 hours and a terminal-phase half-life of about 58.8 hours. However, while the determination of first half-life is sufficiently accurate, the second half-life suffers some inaccuracy, since analysis of plasma levels is less reliable at < 2 ng/ml. At 1–2 hours after the im administration, a steady plasma level is reached and thereafter maintained for the duration of the 7 days (fig 3). Figure 4 shows a scattergram of the correlation between the plasma MAP peak levels observed in 40 patients who received various oral doses.

Linear regression analysis of these data shows a linearity, in the range of 1000–3000 mg, between peak MAP levels and administered dose. The ANOVA (analysis of variance) relative to both the regression procedure and pure error indicates the significance of the linear regression (99% level) (15). A similar procedure

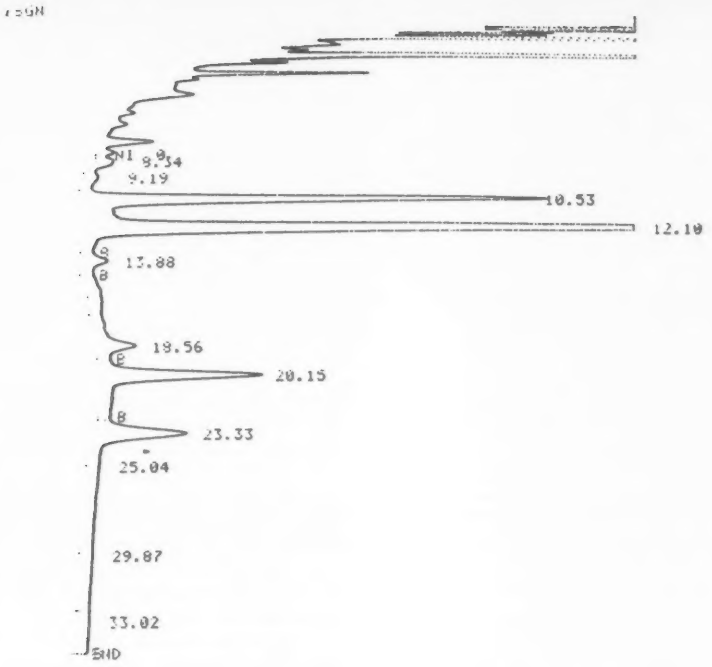

FIGURE 1.—Gas chromatogram showing a typical analysis: 500 mg, im, 26 hrs; MAP level, 5.3 ng/ml (retention time, 13.88 mins); 17α-hydroxyprogesterone caproate (internal standard), 134.2 ng/ml (retention time, 20.15 mins).

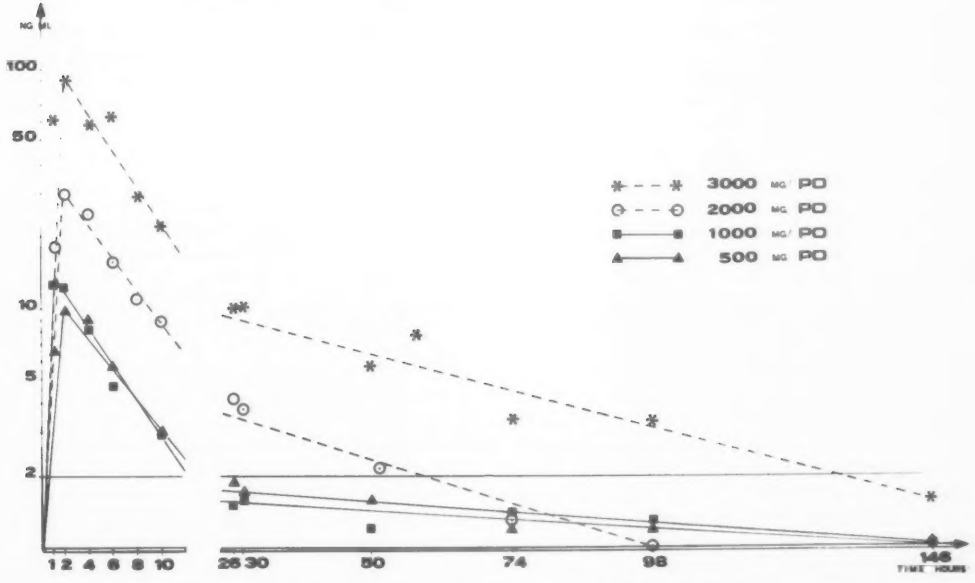

FIGURE 2.—Plasma MAP levels following oral administration. Accuracy of determining MAP levels < 2 ng/ml is low.

TABLE 1.—MAP interpolation of absorption/decay curves (oral administration)*

Parameters	MAP dose							
	500 mg		1000 mg		2000 mg		3000 mg	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
α	–0.77	0.52	–0.88	0.16	–0.64	0.28	0.80	0.33
$t_{1/2}\alpha$	1.05	0.45	0.81	0.16	1.30	0.60	1.00	0.40
β	–0.52	0.14	–0.61	0.22	–0.51	0.17	0.42	0.15
$t_{1/2}\beta$	1.43	0.37	1.25	0.39	1.29	0.26	1.63	0.39
γ	†	†	†	†	–0.015	0.006	–0.011	0.006
$t_{1/2}\gamma$	†	†	†	†	53.2	15.2	64.5	28.6

*$\bar{X}$ = mean of data from individual patients; $t_{1/2}$ = hours.
†Because of the low accuracy of the analytic method at < 2 ng/ml, these data are scarcely significant.

applied to plasma levels after im administration reveals less significant statistical correlation.

The area under the concentration/time curve was calculated for individual patients using the trapezoidal rule in the 0–146-hour time interval. The results (tables 2,3) are in agreement with the data obtained by analytic integration of the interpolated curves in the same time span. Here again, the existence of a statistically significant linear correlation between administered dose and area under the concentration/time curve (0–146 hours) was tested by analysis of variance; in this case a correlation ($P < 0.001$) was found for both oral and im administration.

DISCUSSION

Although the intrinsic low specificity of the radioimmunoassay (RIA) of MAP can now be partially overcome by using a selective extraction technique, we have chosen to use a gas chromatographic method, which assures the complete absence of interferences with MAP metabolites and offers a more accurate run-to-run coefficient of variation and a higher linearity range.

It is of interest to compare the decay curves of MAP plasma concentrations after oral administration determined by gas chromatographic analysis with those reported using RIA techniques (16). In the latter case, peak plasma levels are shifted at longer times after administration, and fairly high amounts of MAP are still observed after 168 hours.

These apparent discrepancies from our data can probably be explained by cross-reactivities with MAP metabolites in the RIA procedure (17). The simplest pharmacokinetic model consistent with the observed plasma decay after oral administration is a two-compartment open model. The first decay phase (table 1) can probably be attributed to the distribution in peripheral tissues and is obscured after im administration as a result of the prolonged absorption. The subsequent "biologic half-life" is considerably longer. Although the

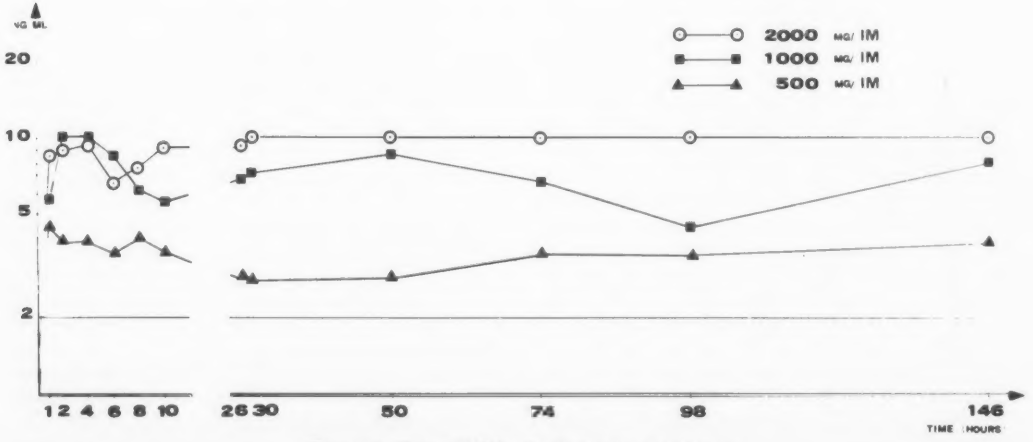

FIGURE 3.—Plasma MAP levels following im administration.

TABLE 2.—MAP areas under absorption/decay curves (ng.h.ml^{-1}) of individual patients (oral administration)

	ng.h.ml^{-1} for MAP dose—			
	500 mg	1000 mg	2000 mg	3000 mg
	126.5	630.0	454.8	229.3
	227.4	381.5	894.4	653.6
	291.6	175.5	382.0	1123.1
	64.3	181.9	587.3	3217.2
	256.9	240.0	400.1	1931.7
	336.2	255.4	141.5	908.7
	336.2	248.0	985.2	829.6
	409.0	160.6	358.6	910.0
	251.9	13.7	296.6	1605.0
	309.9	46.6	125.0	980.2
Mean value	255.0	233.2	442.6	1238.8
± SD	16.0	174.6	306.1	840.3

TABLE 3.—MAP areas under absorption/decay curves (ng.h.ml^{-1}) of individual patients (im administration)

	ng.h.ml^{-1} for MAP dose—		
	500 mg	1000 mg	2000 mg
	404.0	1648.3	668.5
	358.1	2010.7	3960.5
	398.2	1893.4	1096.4
	173.1	1174.3	2702.6
	669.4	386.2	1435.7
	250.5	474.1	1248.9
	470.7	881.6	536.1
	1423.8	299.0	1295.4
	422.8	294.2	638.5
	464.6	853.7	1451.5
Mean value	503.5	991.6	1503.0
± SD	349.4	662.3	1061.3

differences in these half-life parameters at different doses are not statistically significant, the best estimate of the biologic half-life of MAP is probably derived from the 3000-mg (oral administration) curve, which is less affected by the low accuracy of the analytic data < 2 ng/ml.

Some interesting conclusions can be drawn from the data reported in the previous section. MAP is rapidly absorbed after oral administration, and plasma concentration of the drug increases significantly with respective increases in the administered dose. Bioavailability is highly variable (tables 2 and 3 and fig 4), and this must be taken into account when treatment is scheduled. In addition, the high interindividual range in plasma levels indicates the need to improve the absorption efficiency of this drug, especially by testing new dosage forms that are better suited for high-dose oral treatment than the presently used vials optimized for im administration. Preliminary results in our laboratory show that by using 500-mg MAP tablets instead of aqueous suspension as a drug delivery system, both the mean plasma MAP concentrations and the bioavailability are higher. However, the large interindividual variance renders these differences scarcely significant. While im administration results in a better long-term absorption, the short-term bioavailability (0–24 hours) is more favorable after oral treatment.

The present findings have several important areas of clinical application. The knowledge of the dose-dependent MAP peak levels after oral administration, of the peculiar long-term absorption following an im treatment, and of the dose-dependent bioavailability could be relevant in planning any therapeutic use of this drug. In addition, the high interindividual variance in plasma

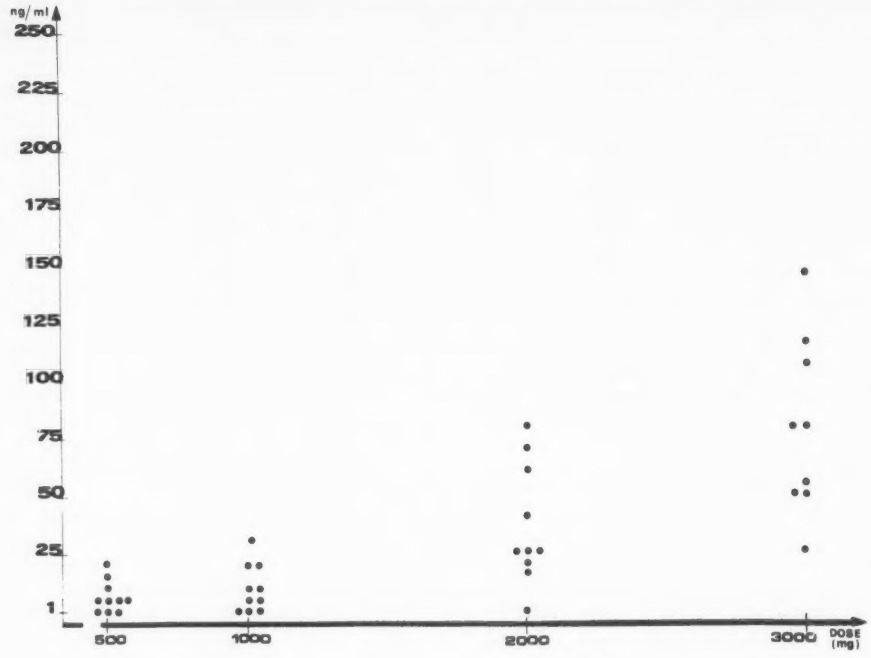

FIGURE 4.—Plasma MAP peak levels in individual patients after oral administration of different doses.

levels indicates that if any significant correlation with clinical response is to be made, the extent of MAP absorption must be determined for each patient.

In an ongoing study we are testing MAP plasma levels following multiple administration. The large inter-subject spread in bioavailability is still present. Hopefully, more clinically significant conclusions will soon be reached.

REFERENCES

1. PANNUTI F, MARTONI A, LENAZ GR, ET AL. A possible new approach to the treatment of metastatic breast cancer: massive doses of medroxyprogesterone acetate. Cancer Treat Rep 62:499–504, 1978.
2. PANNUTI F, MARTONI A, DI MARCO AR ET AL. Prospective, randomized clinical trial of two different high dosages of medroxyprogesterone acetate (MAP) in the treatment of metastatic breast cancer. Eur J Cancer 15:593–601, 1979.
3. PANNUTI F, MARTONI A, FRUET F, ET AL. Hormone therapy in advanced breast cancer: high dose medroxyprogesterone acetate (MAP) vs tamoxifen (TMX)—preliminary results. *In* Breast Cancer. Experimental and Clinical Aspects (Mouridsen HT, and Palshof T, eds). London, Pergamon Press, 1979, pp 93–98.
4. PANNUTI F, MARTONI A, FRUET F, ET AL. Il tumore della Mammella: il trattamento della fase avanzata. *In* La Chemioterapia dei Tumori Solidi, vol 2 (Pannuti F, ed). Bologna, Italy, Patron, 1979, pp 439–509.
5. PANNUTI F, DI MARCO AR, MARTONI A, ET AL. Medroxyprogesterone acetate in treatment of metastatic breast cancer. Seven years of experience. *In* Role of Medroxyprogesterone in Endocrine-Related Tumours (Iacobelli S, and Di Marco A, eds). New York, Raven Press, 1980, pp 73–92.
6. ROBUSTELLI DELLA CUNA G, CALCIATI A, BERNARDO-STRADA MR, ET AL. High dose of medroxyprogesterone acetate (MAP) treatment in metastatic carcinoma of the breast: a dose-response evaluation. Tumori 64:143–149, 1978.
7. DI MARCO A. The antitumor activity of 6α-methyl-17α-acetoxy progesterone (MAP) in experimental mammary cancer. *In* Role of Medroxyprogesterone in Endocrine-Related Tumours (Iacobelli S, and Di Marco A, eds). New York, Raven Press, 1980, pp 1–20.
8. PANNUTI F, ROSSI AP, and PIANA E. Massive doses of medroxyprogesterone acetate (MAP): pilot study in the treatment of advanced prostate cancer. IRCS 5:375, 1977.
9. RENDINA GM, and DONADIO C. La terapia con progestageni nel carcinoma dell'endometrio in fase avanzata. Minerva Ginecol 27:1–5, 1975.
10. PANNUTI F, MARTONI A, ROSSI AP, ET AL. The role of endocrine therapy for relief of pain due to advanced cancer. *In* Advances in Pain Research and Therapy, vol 2 (Bonica JJ, and Ventafridda V, eds). New York, Raven Press, 1979, pp 145–165.
11. NIE NH, ET AL. SPSS Statistical Package for the Social Sciences, 2nd ed. New York, McGraw-Hill, 1980.
12. DIXON WJ, and BROWN MB, eds. BMDP-79. Biomedical Computer Programs. P-Series. Berkeley, University of California Press, 1979.

13. KAISER DG, CARLSON RG, and KIRTON KT. GLC determination of medroxyprogesterone acetate in plasma. J Pharm Sci 63:420–424, 1974.
14. WAGNER JG. Fundamentals of Clinical Pharmacokinetics, 3rd ed. Hamilton, Drug Intelligence Publications, 1975.
15. DRAPER NR, and SMITH H. Applied Regression Analysis. New York, Wiley, 1966, pp 13–32.
16. SALIMSCHIK M, MOURIDSEN HT, LOEBER J, ET AL. Comparative pharmacokinetics of medroxyprogesterone acetate administered by oral and intramuscular routes. Cancer Chemother Pharmacol 4:267–269, 1980.
17. SRIRIMANKER K, SAXENA BN, and FOTHERBY K. A radioimmunoassay for serum medroxyprogesterone acetate. J Steroid Biochem 9:359–363, 1978.

Comparison of Thymidine and Folinic Acid Protection From Methotrexate Toxicity in Human Lymphoid Cell Lines[1,2]

George P. Browman[3,*]

Hypoxanthine, thymidine, and folinic acid protection from methotrexate cytotoxicity were compared in human lymphoid cell lines variably sensitive to thymidine-induced growth suppression. Hypoxanthine protection differed among the cell lines, and the dose-response relationship for protection occurred within the physiologic bone marrow hypoxanthine concentration range. The slopes of the thymidine protection curves were virtually superimposable in a B cell line versus a T cell line, but threefold to fourfold higher thymidine concentrations were required to maximally protect the B versus the T cell line. Thymidine (plus hypoxanthine) protection was superior to that of folinic acid in both the B and the T cell lines when protection was delayed. As the interval between methotrexate and folinic acid exposure was progressively delayed, there was a linear decline in the degree of maximum protection in both cell lines. However, as thymidine exposure was progressively delayed, maximal protection was maintained, except at 12 hours in the B cell line. The influence of methotrexate on thymidine-induced growth suppression was studied. Methotrexate enhanced thymidine-induced growth suppression in sensitive cells as manifested by a significant shift of the dose-response curve. Deoxycytidine protection from thymidine toxicity was superior in the presence of methotrexate. The results of these studies indicate that thymidine protection and folinic acid protection against methotrexate toxicity produce different effects, which in part are dependent on the cell type. The complexity of these interactions points out the need for further studies. [Cancer Treat Rep 66:2051–2059, 1982]

Clinical studies have confirmed that thymidine infusions can protect against methotrexate toxicity in man (1–4). The use of thymidine with methotrexate provides the potential for differential selectivity of rescue between tumor and normal tissues, which could result in a superior antitumor effect. Studies in experimental systems show that protection from methotrexate toxicity requires a salvageable purine as well as thymidine. Therefore, clinical selectivity would depend on: (*a*) the physiologic purine levels, (*b*) the purine salvage capabilities of tumors and normal tissues, and (*c*) the thymidine salvage capabilities of tumors and normal tissues. In addition to determining differential rescue between normal and tumor tissues, these factors might also contribute to the antitumor spectrum of methotrexate.

Certain biochemical properties of human lymphoid cell lines suggest that thymidine utilization might vary among lymphoid subsets of B, T, and null cell origins. Cells with T and null cell characteristics are more sensitive to the growth-inhibitory effects of thymidine than are cells with B cell characteristics (5–8). This differential sensitivity seems to be related to differences of intracellular thymidine metabolism. Thymidine catabolism is more active in B cells, where thymidine phosphorylase levels or the ratio of thymidine phosphorylase to thymidine kinase is higher than in T and null cells (9,10). Furthermore, intracellular clearance of dTTP is more rapid in B cells exposed to high concentrations of thymidine (8,9). Deoxycytidine can reverse thymidine toxicity in vitro (11,12). Therefore, thymidine toxicity is thought to be due to allosteric inhibition of ribonucleotide reductase by excess dTTP, which results in

[1]Received Feb 9, 1982; revised Sept 1, 1982; accepted Sept 21, 1982.
[2]Supported by the National Cancer Institute of Canada.
[3]Ontario Cancer Foundation, Hamilton, Canada.

**Reprint requests to:* George P. Browman, MD, Ontario Cancer Foundation, Hamilton Centre, 711 Concession St, Hamilton, Ontario L8V 1C3, Canada.

decreased conversion of CDP to dCDP (13). This will ultimately deplete intracellular dCTP levels and consequently DNA synthesis.

Methotrexate, a clinically useful drug for the treatment of a variety of lymphoid malignancies, produces a rapid depletion of intracellular dTTP pools (14). We therefore hypothesized that the enhanced thymidine catabolism and rapid clearance of dTTP by B cells might result in increased requirements for thymidine to protect against methotrexate toxicity. The in vitro studies reported here were designed to test thymidine requirements for methotrexate protection among lymphoid cell lines variably sensitive to thymidine-induced growth inhibition. Thymidine protection was compared to folinic acid protection, and we also examined the interaction of toxic concentrations of thymidine with methotrexate.

Since selective thymidine protection or rescue in vivo might also be influenced by ability to utilize circulating salvageable purines, we also studied hypoxanthine requirements for methotrexate protection among these cells.

MATERIALS AND METHODS

Human lymphoblastoid cell lines were provided by Dr. Herbert Lazarus of the Sidney Farber Cancer Institute (Brookline, MA) and Dr. Jun Minawada of Roswell Park Memorial Institute (Buffalo) and were maintained in suspension culture in RPMI 1640 tissue culture medium (Grand Island Biological Co [GIBCO], Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO), 20 mM HEPES buffer (Sigma Chemical Co, St. Louis), penicillin (100 units/ml) (GIBCO), and streptomycin (100 μg/ml) (GIBCO).

Methotrexate and folinic acid were obtained from Cyanamid Canada, Inc (Willowdale, Ontario), and thymidine, deoxycytidine, and hypoxanthine were obtained from Sigma Chemical Co. Thymidine requirements for methotrexate protection were assessed during a 72-hour growth period in which 2×10^5 cells in triplicate 1-ml cultures were exposed to a maximum inhibitory concentration of methotrexate (0.2–1.0 μM), a fixed concentration of hypoxanthine (80 μM), and varying concentrations of thymidine. The hypoxanthine concentration was determined in preliminary experiments, and hypoxanthine was added to cultures at the same time as methotrexate. Varying concentrations of thymidine were added to cultures under separate conditions, at the same time as methotrexate or at 1, 4, 6, or 12 hours after methotrexate to allow a progressive fall in dTTP levels. Hypoxanthine requirements for methotrexate protection were assessed in the same way as for thymidine except that the thymidine concentration was fixed and the hypoxanthine concentration was varied. Hypoxanthine protection was measured only with simultaneous exposure to methotrexate. For these experiments, medium was supplemented with extensively dialyzed fetal bovine serum. At the end of the incubation (72 hours after addition of thymidine or hypoxanthine), the cells were electronically counted in a Coulter Counter model ZF. Growth in treated cultures is expressed as a percent of the growth achieved in 72-hour control cultures without additives. The abilities of thymidine and hypoxanthine to protect against methotrexate, when the compounds were added simultaneously, is expressed as a PC_{50} value, which represents the concentration at which 50% of maximum protection is achieved, assuming zero protection as the effect of methotrexate alone.

Folinic acid protection was also measured to control for differences in thymidine protection between B and T cells that were unrelated to thymidine metabolism. For these experiments, the maximum protection provided by selected concentrations of folinic acid (described in Results section) was assessed 72 hours after folinic acid was added to methotrexate-treated cultures.

Thymidine toxicity was measured in the presence and absence of methotrexate by evaluating its growth-inhibitory effect at varying concentrations over a 72-hour exposure period. Growth inhibition is quantitatively expressed as an IC_{50} value, which corresponds to the thymidine concentration that inhibits growth to 50% of that of untreated cultures. Deoxycytidine reversal experiments were performed by adding the compound to cultures simultaneously with thymidine.

All cultures were documented to be free of mycoplasma contamination through serial determinations using the differential uridine:uracil incorporation method of Schneider et al (15) and the mycoplasma culture test kit provided by Flow Laboratories (McLean, VA). The growth rates of all cell lines were similar.

RESULTS

The maximum cytotoxic effect of methotrexate was reached in all cells at a concentration of 0.05 μM (data not shown). Simultaneous thymidine and hypoxanthine protection were assessed at a 1.0-μM concentration of methotrexate, while delayed protection with thymidine and folinic acid was assessed at a 0.2-μM methotrexate level.

Thymidine dose-response inhibition curves are depicted in figure 1. They confirm the enhanced thymidine sensitivities of the malignant T (CCRF-CEM and MOLT-4) and malignant null (LAZ 221) cell lines relative to the Epstein-Barr virus-transformed "normal" B cell line (LAZ 007). The differences in thymidine sensitivities are quantitatively expressed as IC_{50} values in the legend. Figure 2 shows the dose-response relationship for the protective effect of hypoxanthine in all the cell lines.

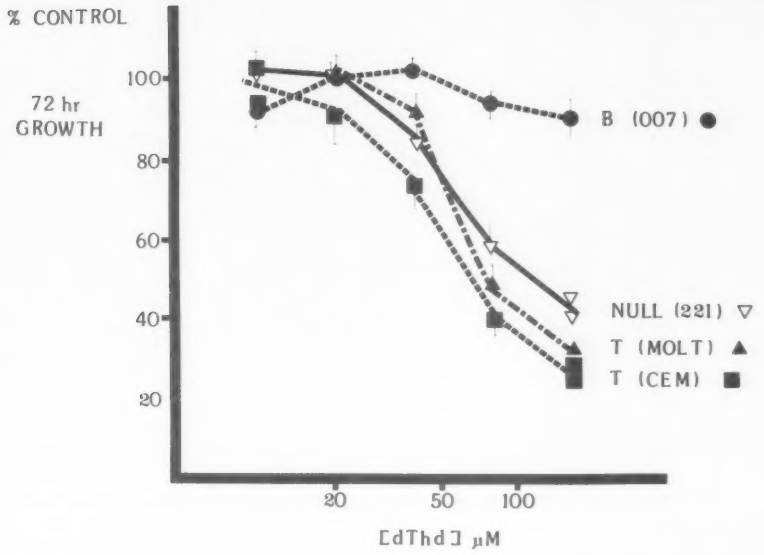

FIGURE 1.—Thymidine (dThd) dose-response toxicity curves in lymphoid cell lines. Cells in suspension were exposed in triplicate to varying thymidine concentrations for 72 hrs and then counted; growth is expressed as a % of control. Error bars = SE from at least 4 separate experiments. IC_{50} values (mean ± SE): LAZ 221 = 104 ± 10 μM; MOLT-4 = 82 ± 7 μM; CCRF-CEM = 51 ± 3 μM.

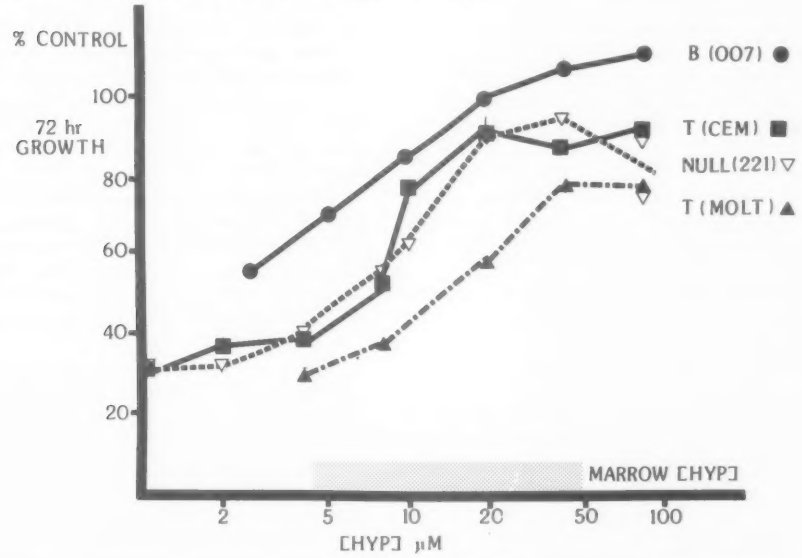

FIGURE 2.—Hypoxanthine (HYP) dose-response protection curves from methotrexate cytotoxicity. Cells were exposed in triplicate to varying hypoxanthine concentrations in the presence of 1.0 μM methotrexate and 20–40 μM thymidine for 72 hrs and then counted. Results are expressed as a % of control growth. Shaded area along abscissa corresponds to bone marrow hypoxanthine concentration range measured by Howell et al (16). Error bars = SE from at least 4 separate experiments. PC_{50} (mean ± SE): LAZ 007 = 6.2 ± 2.4 μM; LAZ 221 = 9.1 ± 1.4 μM; MOLT-4 = 15.2 ± 3.1 μM; CCRF-CEM = 8.5 ± 1.0 μM.

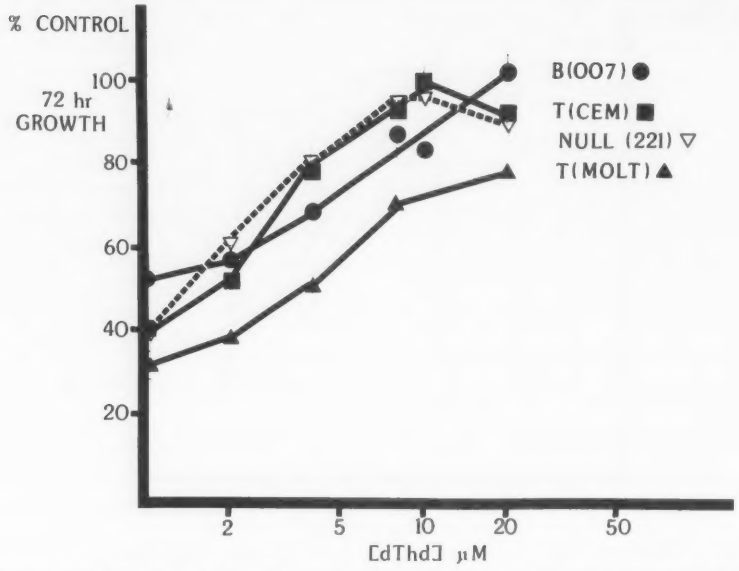

FIGURE 3.—Thymidine (dThd) dose-response protection curves from methotrexate cytotoxicity. Cells were exposed in triplicate to varying thymidine concentrations in the presence of 1.0 μM methotrexate and 80 μM hypoxanthine for 72 hrs and then counted. Results are expressed as a % of control growth. Error bars = SE from at least 4 separate experiments. PC_{50} (mean ± SE): LAZ 007 = 4.0 ± 0.9 μM; LAZ 221 = 2.2 ± 0.2 μM; MOLT-4 = 3.8 ± 0.5 μM; CCRF-CEM = 2.2 ± 0.3 μM.

The hypoxanthine requirements for methotrexate protection are quantitatively expressed as PC_{50} values in the legend. It is clear that hypoxanthine requirements vary among the cell lines. Of particular interest is the location of the steepest portion of all the dose-response curves, which falls within the physiologic concentration range for hypoxanthine as measured by Howell et al (16). This implies that small changes in hypoxanthine concentration may significantly influence methotrexate growth inhibition.

Figure 3 shows a dose-response relationship for thymidine protection in all cell lines when thymidine is added at the same time as methotrexate. The curves for CCRF-CEM (T cell) and LAZ 221 (null cell) are virtually superimposed. The absolute thymidine requirement is greatest for MOLT-4, as reflected by the rightward shift of the protection curve. However, the slope of the curve does not differ from that of the other cell lines. The thymidine dose-requirement curve for the B cell line LAZ 007 falls in an intermediate position. Although its slope is slightly flatter than those for the other cell lines, this difference is small. The relative thymidine requirements for methotrexate protection are expressed quantitatively as PC_{50} values in the legend. The results indicate that thymidine requirements for methotrexate protection measured in this way are not influenced by B, T, or null cell status.

Since the interaction between methotrexate and thymidine has been shown to be sequence-dependent (17), we extended these studies by prolonging the time interval between methotrexate and subsequent thymidine exposure in the B cell line (LAZ 007) and one T cell line (CCRF-CEM). We hypothesized that the delay in thymidine exposure would allow a progressive fall in the intracellular dTTP pool, thus unmasking any differences in thymidine utilization that were not apparent when thymidine and methotrexate were added simultaneously to cultures. A significant fall in dTTP levels would be expected within 4 hours of methotrexate exposure (14).

The thymidine dose-protection curves in these delay experiments were superimposed at all time points. Thus, a time-related shift in thymidine requirements for protection was not observed for either cell line up to 12 hours.

To further define measurable differences in the efficiency of thymidine protection, we calculated the mean maximum 72-hour growth achieved following each delay period and the mean thymidine concentration required to provide maximum growth (table 1). Threefold to fourfold more thymidine was required for maximum protection of the B cells versus the T cells at time points prior to 12 hours. At 12 hours, the difference in thymidine requirements between the cell lines was smaller

but still significant. At the minimum thymidine concentration that maximally protected the B cells (40 μM), substantial toxicity was already observed in the CCRF-CEM cells, as shown in figure 5.

We compared maximum protection offered by thymidine and a single concentration of folinic acid by observing their abilities to support maximum growth in B (LAZ 007) and T (CCRF-CEM) cells. A single folinic acid concentration was studied since the intent was to use this form of rescue to control for differences not related to thymidine metabolism. The folinic acid concentration chosen (16 μM) was approximately 100 times the molar equivalent of methotrexate (0.2 μM). We had previously found, using another cell line, that almost complete protection could be achieved over a 72-hour growth period at this folinic acid:methotrexate concentration ratio (18).

The results comparing maximum thymidine and folinic acid protection (table 1) are graphically illustrated in figure 4. Folinic acid and thymidine (plus hypoxanthine) protection were almost complete (~ 100% of control growth) at zero time, when the rescue agents and methotrexate were added simultaneously to the cultures. As the folinic acid delay interval was progressively prolonged, there was a linear decline in the extent of protection in both cell lines. Folinic acid protection was superior in the B versus the T cell line at all time points.

As the thymidine delay interval was progressively prolonged, no decline in protection occurred until 12 hours in the B cell only. At the later time points thymidine (plus hypoxanthine) offered better protection than folinic acid in both cell lines.

The influence of methotrexate on thymidine-induced growth suppression was studied in the presence of hypoxanthine and escalating concentration of thymidine past the point of maximum protection. The methotrexate effect was similar for all cells and only that for CCRF-CEM is shown graphically (fig 5). Methotrexate added simultaneously with thymidine shifted the toxicity curve to the left, suggesting sensitization of the cells to thymidine. This sensitization is quantitatively expressed by comparing IC_{50} values for thymidine with and without methotrexate (*see* fig 5 legend). It is unclear why thymidine-induced growth suppression should be enhanced by simultaneous exposure to methotrexate. Simultaneous thymidine exposure may prevent a significant methotrexate-induced fall of dTTP levels by bypassing the de novo block in thymidylate synthesis. However, the rise in dTTP levels would not be expected to exceed the rise produced when exogenous thymidine is added without methotrexate.

In experiments in which thymidine exposure is delayed, a fall in intracellular dTTP levels after methotrexate treatment would be expected to shift the thymidine toxicity curve to the right since more thymidine would be required to attain an equivalent dTTP level. However, even under these circumstances, there is no evidence of an increased thymidine dose requirement to produce equivalent toxicity. Further discussion of these observations in light of the proposed mechanisms of methotrexate- and thymidine-induced growth suppression is presented below.

To evaluate the relative contribution of dCTP depletion to thymidine-induced toxicity in the presence and absence of methotrexate, we compared deoxycytidine

TABLE 1.—Comparison of thymidine and folinic acid protection in a B cell line (LAZ 007) and a T cell line (CCRF-CEM)*

Time (hrs)†	Cell type	Maximum protection: % of control growth		Thymidine concentration (μM) for maximum protection
		Thymidine	Folinic acid	
0	B	97 ± 2	102 ± 2	33 ± 10
	T	92 ± 2	90 ± 3	9.4 ± 0.3
1	B	98 ± 3	101 ± 3	32 ± 4
	T	91 ± 2	92 ± 1	9.1 ± 1.0
4	B	102 ± 7	95 ± 5	40 ± 16
	T	97 ± 2	83 ± 3	10 ± 0
6	B	103 ± 3	—	40 ± 8
	T	100 ± 2	83 ± 3	9.6 ± 1.0
12	B	89 ± 8	84 ± 3	26 ± 7
	T	104 ± 3	67 ± 5	12 ± 2

*Values = mean ± SE.
†From addition of methotrexate to thymidine or folinic acid exposure.

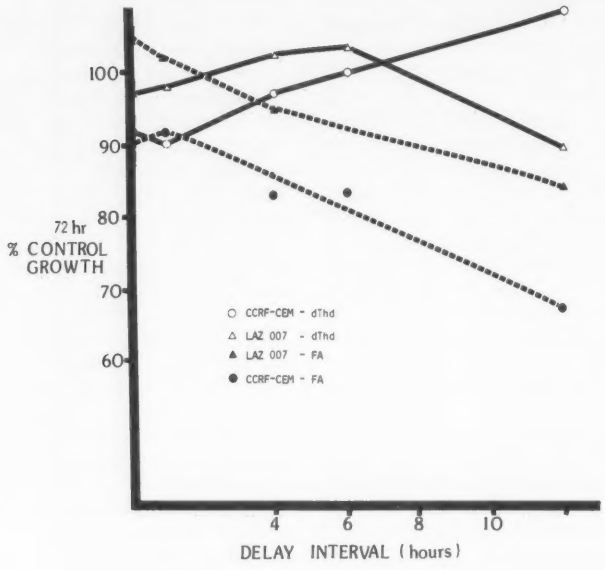

FIGURE 4.—Comparison of maximum protection from methotrexate toxicity offered by thymidine (dThd) and folinic acid (FA) in CCRF-CEM and LAZ 007. Cells were exposed in triplicate to 0.2 μM methotrexate, and for thymidine protection, 80 μM hypoxanthine was included. At 0, 4, 6, or 12 hrs after methotrexate exposure, 16 μM folinic acid or optimal concentrations of thymidine were added, and 72 hrs later cells were counted. Results are expressed as a % of control growth. Values for thymidine protection were calculated from the thymidine delay experiments described in the text. Error bars = SE from at least 4 separate experiments.

reversal under these conditions. The results (table 2) indicate that at 80 μM thymidine, a given concentration of deoxycytidine will provide excellent protection from toxicity in the presence and absence of methotrexate, although reversal is superior with methotrexate present. Studies in the murine system indicate that circulating deoxycytidine may be an important factor determining thymidine effects in vivo (19).

DISCUSSION

The studies described here were initiated to explore an in vitro model in which known differences in thymidine metabolism would be expected to differentially influence methotrexate toxicity during thymidine rescue. To control for factors that might contribute to differences in protection that were independent of thymidine metabolism, we compared the efficiency of thymidine and folinic acid rescue. We also studied the efficiency of hypoxanthine rescue and the influence of methotrexate on thymidine-induced growth suppression since these variables might also influence in vivo effects of thymidine and methotrexate treatment.

These studies failed to demonstrate significant differences in thymidine utilization for methotrexate protection among cells with widely different rates for thymidine catabolism except at concentrations needed for maximum protection. The thymidine concentration that maximally protects a B cell line (30–40 μM) is threefold to fourfold higher than that needed to protect a T cell line. However, this difference occurs at a thymidine level that provides substantial growth inhibition in all the T cell lines and is far above the in vivo level needed to protect against methotrexate toxicity (1,2,4). The net result of these effects is a substantial difference in growth between the cell lines, which is more closely related to thymidine suppression than to thymidine protection.

Substantial differences were observed between thymidine and folinic acid protection in the cell lines studied. While thymidine offered similar protection to both cell lines (except at 12 hours), folinic acid offered better protection to LAZ 007 than to CCRF-CEM at all time points. Secondly, the degree of protection offered by these agents in each individual cell line was quite different. In LAZ 007, thymidine and folinic acid produced

TABLE 2.—Deoxycytidine reversal of thymidine-induced growth inhibition in CCRF-CEM cells in presence and absence of methotrexate (+ hypoxanthine)

Drug	% of control growth, mean ± SE	
	Without deoxycytidine	With 80 μM deoxycytidine
Thymidine alone (80 μM)	25 ± 3	80 ± 1
Thymidine (80 μM) + methotrexate (0.2 μM)*		
0 hr†	22 ± 1	93 ± 4
1 hr	26 ± 2	93 ± 2
6 hr	25 ± 1	88 ± 3

*Growth in 0.2 μM methotrexate alone = −15% ± 1% of control growth.
†Delay interval between exposure to methotrexate (+ hypoxanthine) and addition of thymidine.

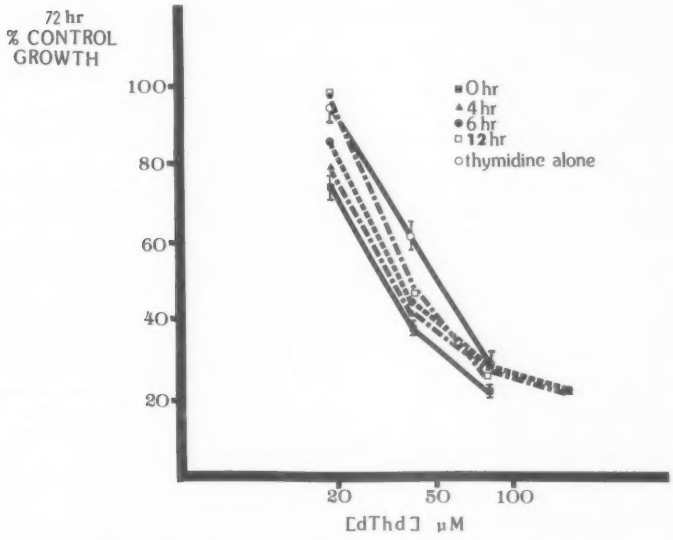

FIGURE 5.—Thymidine (dThd) dose-response cytotoxicity curves in the presence and absence of methotrexate. CCRF-CEM cells in triplicate were exposed to varying concentrations of thymidine alone or to 0.2 μM methotrexate. In methotrexate-treated cultures, 80 μM hypoxanthine was included, and varying concentrations of thymidine were added at 0, 4, 6, or 12 hrs after methotrexate exposure. Cells were counted 72 hrs after thymidine exposure and results are expressed as a % of control growth. Points represent mean values from at least 5 separate experiments. Error bars = SE. IC_{50} (mean ± SE): thymidine alone = 51 ± 3 μM; thymidine + methotrexate (0 hr) = 34 ± 3 μM.

equivalent protection, while in CCRF-CEM, thymidine protection was vastly superior to folinic acid protection. Such differences might be explained by cellular differences in susceptibility to an early methotrexate-induced purineless state (20), since hypoxanthine was included in thymidine-treated but not in folinic acid-treated cultures, or by reported differences in folate catabolism between malignant and nonmalignant cells (21). Further studies are in progress to characterize the differences in folinic acid protection seen with these cell lines.

In contrast to the observations on thymidine protection, differences in hypoxanthine protection among cells and the steep portions of the hypoxanthine protection curves did occur at physiologic hypoxanthine concentrations, indicating that small differences in hypoxanthine concentration might have profound effects on cell growth. Since blood and bone marrow hypoxanthine levels differ significantly among individuals (16), the

selective antitumor effects of methotrexate during thymidine rescue may be closely related to purine levels and purine salvage capability. Studies in the murine system support a role for endogenous purines in modulating in vivo effects of methotrexate (22).

The clinical use of methotrexate and thymidine in combination might be considered for tumors sensitive to both agents. Therefore, we examined the effects of methotrexate (plus hypoxanthine) on thymidine-induced growth inhibition. Thymidine inhibition depends upon an expanded dTTP pool while methotrexate depletes this pool. We thus expected that in the presence of methotrexate, excess thymidine might be required to produce toxic effects equivalent to those produced by thymidine alone. This might be especially true in circumstances in which thymidine exposure is delayed, since a progressive fall in intracellular dTTP pools would occur prior to addition of thymidine. However, the opposite phenomenon was observed (fig 5); the thymidine suppression curve was shifted to the left in the presence of methotrexate. The observations on deoxycytidine reversal of thymidine toxicity in the presence and absence of methotrexate may provide a clue to the explanation of thymidine sensitization by methotrexate. Deoxycytidine (which has no effect on methotrexate growth suppression in the absence of thymidine) substantially reversed thymidine toxicity both in the presence and absence of methotrexate (table 2). This suggested that the toxicity observed in the presence of methotrexate was in large part due to thymidine. However, methotrexate will also cause a decline of dCTP pools, which is thought to be responsible for thymidine toxicity. This methotrexate effect depends upon dTTP depletion with subsequent release of its natural inhibition on dCMP deaminase. This in turn allows more dCMP to be converted to dUMP rather than dCTP (23). Thus, a paradoxical situation exists in which dCTP depletion in the presence of methotrexate depends upon *low* dTTP levels, while in the presence of thymidine, *high* dTTP levels result in dCTP depletion. The superior reversal of thymidine toxicity by deoxycytidine in the presence of methotrexate is unexplained but could be related to changes in purine deoxynucleoside triphosphate pool sizes in the presence and absence of methotrexate. Modulation of purine ribonucleotide reductase by dTTP results in enhanced dGDP formation and elevated dGTP pools. Grindey et al (12,24) have argued that elevated dGTP pools may contribute to thymidine toxicity through modulation of a regulatory protein for DNA polymerase. Thus, thymidine may inhibit DNA synthesis, and subsequently cell growth, through depletion of dCTP as well as through elevation of dGTP. However, the addition of methotrexate, which has an antipurine effect, may prevent dGTP from rising to inhibitory levels, especially in the presence of a low concentration of salvageable purine. Therefore, in the presence of methotrexate, dCTP depletion would play a proportionally larger role in inhibiting cell growth, and deoxycytidine reversal would be more efficient. In a similar manner, the modulation of thymidine toxicity by methotrexate could be influenced by the concentration of salvageable purine present, since this would influence the size of the dGTP pool and its relative contribution to thymidine toxicity. Measurement of dNTP pool sizes will be required to substantiate such mechanisms.

The results of the in vitro studies reported here point out the complexities that accompany attempted modulation of drug effects.

REFERENCES

1. Ensminger WD, and Frei E, III. The prevention of methotrexate toxicity by thymidine infusions in humans. Cancer Res 37:1857–1863, 1977.
2. Howell SB, Ensminger WD, Krishan A, et al. Thymidine rescue of high-dose methotrexate in humans. Cancer Res 38:325–330, 1978.
3. Howell SB, and Tamerius RK. Achievement of long duration methotrexate exposure with concurrent low dose thymidine protection: influence on methotrexate pharmacokinetics. Eur J Cancer 16:1427–1432, 1980.
4. Howell SB, Herbst K, Boss GR, et al. Thymidine requirements for the rescue of patients treated with high-dose methotrexate. Cancer Res 40:1824–1829, 1980.
5. Schachtschabel DO, Lazarus H, Farber S, et al. Sensitivity of cultured human lymphoblasts (CCRF-CEM cells) to inhibition by thymidine. Exp Cell Res 43:512–514, 1966.
6. Lazarus H, Barell EF, Oppenheim S, et al. Divergent properties of two human lymphocytic cell lines isolated from a single specimen of peripheral blood. In Vitro 5:303–310, 1974.
7. Fox RM, Piddington SK, Tripp EH, et al. Thymidine sensitivity of cultured leukemic lymphocytes. Lancet 2:391–393, 1979.
8. Fox RM, Tripp EH, Piddington SK, et al. Sensitivity of leukemic human null lymphocytes to deoxynucleosides. Cancer Res 41:3383–3386, 1980.
9. Carson DA, Kaye J, Matsumoto S, et al. Biochemical basis for the enhanced toxicity of deoxyribonucleosides toward malignant human T cell lines. Proc Natl Acad Sci USA 76:2430–2433, 1979.
10. Kufe DW, Beardsley P, Karp D, et al. High dose thymidine infusions in patients with leukemia and lymphoma. Blood 55:580–589, 1980.
11. Fox RM, Tripp EH, and Tattersall MHN. Mechanism of deoxycytidine rescue of thymidine toxicity in human T-leukemic lymphocytes. Cancer Res 40:1718–1721, 1980.
12. Grindey GB, Wang MC, and Kinahan JJ. Thymidine induced perturbations in ribonucleoside and deoxyribonucleoside triphosphate pools in human leukemic CCRF-CEM cells. Mol Pharmacol 16:601–606, 1979.
13. Moore EC, and Hurlbert RB. Regulation of mammalian deoxyribonucleotide biosynthesis by nucleotides as activators and inhibitors. J Biol Chem 241:4802–4809, 1966.
14. Fridland A. Effect of methotrexate on deoxynucleotide pools and DNA synthesis in human lymphocytic cells. Cancer Res 34:1883–1888, 1974.
15. Schneider EL, Stanbridge EJ, and Epstein CJ. Incorporation of ^{3}H-uridine and ^{3}H-uracil into RNA. A simple technique for the detection of mycoplasma contamination of cultured cells. Exp Cell Res 84:311–318, 1974.

16. Howell SB, Mansfield SJ, and Taetle R. Thymidine and hypoxanthine requirements of normal and malignant human cells for protection against methotrexate cytotoxicity. Cancer Res 41:945–950, 1981.
17. Jackson RC. Modulation of methotrexate toxicity of thymidine: sequence-dependent biochemical effects. Mol Pharmacol 18:281–286, 1980.
18. Browman GP, Calvert AH, Taylor GA, et al. The intracellular mechanism of action of metoprine (DDMP). Eur J Cancer 16:1547–1554, 1980.
19. Eagen EM, Sargent L, Rosowsky A, et al. Rescue of thymidine cytotoxicity in L1210 ascites by elevated endogenous levels of deoxycytidine. Cancer Treat Rep 65:853–860, 1981.
20. Hryniuk WM. Purineless death as a link between growth rate and cytotoxicity by methotrexate. Cancer Res 32:1506–1511, 1972.
21. Stea B, Backlund PS, Berkey PB, et al. Folate and pterin metabolism by cancer cells in culture. Cancer Res 38:2378–2384, 1978.
22. Browman GP, and Csullog GW. Modification of in vivo methotrexate antitumor effect in L1210 leukemia by induced impairment of purine salvage. Biochem Pharmacol 30:869–874, 1981.
23. Maley F, and Maley GF. On the nature of a sparing effect by thymidine on the utilization of deoxycytidine. Biochemistry 1:847, 1962.
24. Steinberg JA, and Grindey GB. Isolation of a regulatory protein associated with calf thymus DNA polymerase α modulated by deoxyguanosine triphosphate. Fed Proc 38:484, 1979.

Activity of 4′-Epi-doxorubicin on Normal Hematopoietic Precursor Cells in Mice[1,2]

Alberto Sobrero,* Carlo Muzzulini, Francesco D'Amore, Giuseppe Bogliolo, Giulio Massa, Riccardo Ghio, and Ivo Pannacciulli[3,4]

The effects of 4′-epi-doxorubicin (4′-epi-DX) on survival of in situ murine bone marrow hematopoietic precursor cells (HPC) were studied. The drug was given to (C57BL × C3H)F_1 mice in increasing single doses, in repeated administrations (three times in 5 days), or for a long period (twice a week for 10 weeks). Survival of colony-forming units (CFU_s, spleen; CFU_c, culture; and CFU_e, erythroid) was assayed by in vivo and in vitro methods; dose survival curves were exponential. It appears that single doses of 4′-epi-DX have a greater impact on murine HPC than single doses of the parent compound. Following repeated administrations, pluripotential stem cells recover better after the derivative than after its parent drug. During prolonged treatment, the bone marrow content of committed progenitors is depleted to a lesser extent in the animals treated with 4′-epi-DX than in those receiving DX. It is, however, possible that, following the same prolonged treatment, this difference is minimal. In conclusion, these experimental data seem to indicate that 4′-epi-DX has lower toxicity in murine HPC than DX and that this is evident mainly after repeated administrations for a short period of time. [Cancer Treat Rep 66:2061–2070, 1982]

The cytotoxic effect of doxorubicin (DX) on normal hematopoietic cells, together with its serious cardiac toxicity, is a major problem in the clinical use of this drug. Various anthracycline derivatives (1–6) are being studied in an effort to find a compound with an increased therapeutic index. 4′-Epi-doxorubicin (4′-epi-DX) appears to be of particular interest, since both in experimental and clinical studies (7–13) it has shown antitumor activity at least equal to that of the parent compound and has had somewhat lower myocardial and hematologic toxicity. Recently, Lohrmann and Schreml (14) reported several studies on the effects of anthracyclines on mouse hematopoietic precursor cells (HPC). Marsh (15) has shown that the sensitivity of bone marrow hematopoietic progenitors to DX is different from their sensitivity to *N*-trifluoro-acetyladriamycin-14-valerate, another analog of DX, and that this difference may vary according to the animal species studied. In a recent study (16) we compared the effects of DX to those of 4-demethoxydaunorubicin on murine hematopoietic stem cells. In light of this and previous research (17,18) it seems that the assay of hematopoietic stem cells in animals treated with new anthracycline analogs is a useful means for assessing their potential myelotoxicity. The purpose of the study reported here was to evaluate the effects of 4′-epi-DX on survival of in situ bone marrow stem cells following the injection of increasing single doses of the drug or during its repeated or prolonged administration.

MATERIALS AND METHODS

Mice

All experiments were performed on (C57BL♂ × C3H♀)F_1 adult mice, 8–12 weeks old. Recipient mice were irradiated with a Theratron Junior cobalt-60 machine (80 rads/minute; total dose, 900 rads).

[1]Received Mar 10, 1981; revised July 9, 1982; accepted Sept 2, 1982.

[2]Supported in part by CNR contracts 15/79.00653.96/115.4751 and 101/78.02866.96/115.4823.

[3]Cattedra di Patologia Speciale Medica RR (*A. Sobrero, C. Muzzulini, F. D'Amore, G. Bogliolo, G. Massa,* and *I. Pannacciulli*) and Cattedra di Terapia Medica (*R. Ghio*), Istituto Scientifico di Medicina Interna, University of Genoa, Italy.

[4]We thank Prof. A. M. Casazza for helpful suggestions and criticism, Mr. Kenneth Dinnen for preparation of the manuscript, and Ms. B. Johnston for editing the manuscript.

**Reprint requests to:* Dr. Alberto Sobrero, Istituto Scientifico di Medicina Interna, University of Genoa, Viale Benedetto XV 6, 16132 Genoa, Italy.

Drugs

DX and 4′-epi-DX (IMI 28, batch 10024030) hydrochlorides were supplied by Farmitalia-Carlo Erba (Milan, Italy) in the form of red powder. They were stored in the dark in a desiccator at 4°C and dissolved in normal saline (NaCl, 0.9%) at the working concentration immediately before use. Structure, molecular weight, and biologic and pharmacologic activities of 4′-epi-DX have already been reported (7,8,19,20). Both DX and 4′-epi-DX were injected into the tail veins of mice. Only in the case of prolonged administration (10 weeks) were the drugs given ip.

Experimental Project

To fully evaluate the hematologic toxicity of 4′-epi-DX a comparison was made with the parent drug DX. According to previous experimental evidence (7–9), DX and 4′-epi-DX have the same antitumor effect at the same dose; 4′-epi-DX, however, has a much higher therapeutic index than DX. Throughout this study, which was aimed at assessment of hematologic toxicity, comparison between the two anthracyclines was made at equipotential but not equitoxic doses, that is, at equal doses of each drug.

Experiment A: in vivo sensitivity of murine HPC to 4′-epi-DX.—The aim of this experiment was to evaluate the shape and slope of in vivo dose-survival curves of colony-forming units (CFU_s, spleen; CFU_c, culture; and CFU_e, erythroid) in mice treated with 4′-epi-DX. Statistical evaluation of the results allows a comparison between the different levels of toxicity of 4′-epi-DX on the different hematopoietic populations. Groups of five mice each received single iv injections of 4′-epi-DX at various dose levels: that is, 1.6, 2.7, 4.5, 6.8, 11.5, 19.0, and 31.6 mg/kg of body weight. These doses (each $^5/_3$ of the previous one) were selected, taking into account that the LD50 in healthy CDF_1 mice at 1 month for 4′-epi-DX is 18.52 mg/kg (7). Twenty-four hours after injection, the mice were bled from the orbital sinus for determination of packed rbcs, the total wbc count per liter, and the number of reticulocytes per 100 rbcs; then they were killed by cervical dislocation. Both femurs were removed, bone marrow (bm) cellularity was estimated, and bm cell suspensions for HPC assays were prepared. Results were compared with those obtained in matched groups of mice that received equal doses of DX and that were treated and assayed in exactly the same way. These results have already been partially reported (16). Normal, untreated controls injected only with saline were also tested.

Experiment B: time-survival curves for bone marrow HPC in mice treated with repeated doses of 4′-epi-DX.—According to Casazza (1) a schedule of intermittent treatment with 4.5 mg/kg of DX at time 0 and on Days 2 and 5 was particularly useful for enhancing the antileukemic activity of DX in Gross' leukemia-bearing C3H mice. This schedule was applied to define all possible differences in hematologic toxicity between 4′-epi-DX and DX when the two drugs are administered in repeated doses. Accordingly, two groups of 50 mice received 4.5 mg/kg iv of either DX or 4′-epi-DX at time 0 and on Days 2 and 5. On Days 2, 3, 5, 6, 8, 9, 12, 15, 18, and 21, five treated mice from each group and five saline-treated controls were killed shortly after the determination of the above-mentioned peripheral blood cell counts. The assays for progenitor cells were performed on bm cell suspensions.

Experiment C: time-survival curves for bone marrow HPC in mice during long-term administration of 4′-epi-DX.—Long-term administration of anthracyclines leads to a severe cardiotoxic effect. The minimal cumulative cardiotoxicity of 4′-epi-DX, however, is higher than that of DX (8). The aim of this experiment was to see whether equal cumulative doses of 4′-epi-DX and DX have different hematologic toxic effects. Accordingly, two groups of 80 mice were treated twice a week ip with 2.5 mg/kg either of DX of 4′-epi-DX, given for 10 weeks. At the end of each week, the determinations on peripheral blood and bm described above were performed in five treated mice from each group and in five saline-treated controls.

Methods

The peripheral blood cell counts were carried out by conventional methods (21). Bm collection and preparation of single-cell suspensions for CFU assays were performed by the usual techniques (22).

Assays for hematopoietic CFU.—A first aliquot of the cell suspension was assayed for CFU_s content (23). Nine mice, irradiated as reported above, were used as recipients. A total of 0.5 ml of cell suspension, diluted in medium to contain 4×10^4 bm cells, was injected into tail vessels. The host mice were killed with ether 9 days after the injection, and their spleens were excised and fixed in Tellyesniczky's fluid. The number of visible colonies was then counted. The mean number of colonies in each spleen was calculated, and the total number of CFU_s per femur ± SE was determined. A second aliquot of the cell suspension was assayed for CFU_c content (22). One ml of agar α-medium containing 1×10^5 bm cells and 20% horse serum was pipetted into triplicate 35-mm Falcon Petri dishes over 0.1 ml of colony-stimulating factor prepared from an extract of the uterine membrane of a pregnant mouse. The cultures were incubated at 37°C in a 95%–100% humidified atmosphere of 5% CO_2 in air. Cultures were examined after 7 days of incubation, using an inverted microscope. The number of colonies containing > 50 cells was

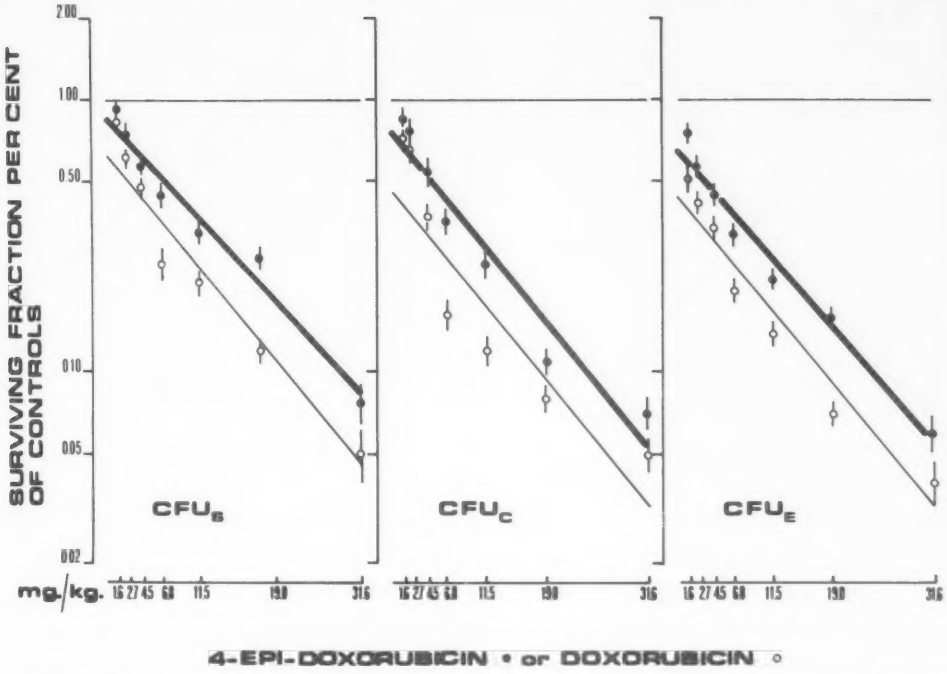

FIGURE 1.—Dose-survival curves of bm HPC 24 hrs after 4′-epi-DX or DX single injection. For each experimental point, vertical bars = mean ± SE (normal values/femur in untreated mice: CFU_s = 3934 ± 92; CFU_c = 14,162 ± 635; CFU_e = 14,560 ± 654). 4′-Epi-DX: CFU_s, r = –0.984 ($P < 0.01$); CFU_c, r = –0.968 ($P < 0.01$); CFU_e, r = –0.979 ($P < 0.01$). DX: CFU_s, r = –0.967 ($P < 0.01$); CFU_c, r = –0.968 ($P < 0.01$); CFU_e, r = –0.973 ($P < 0.01$).

recorded, and the mean number of CFU_c per femur ± SE was calculated. A third aliquot of the same cell suspension was assayed for its CFU_e content (24). A total of 1×10^5 nucleated bm cells in 1 ml of a mixture containing 0.8% methylcellulose, 30% horse serum, and 0.25 units of erythropoietin were plated into triplicate 35-mm Falcon Petri dishes. The erythropoietin was prepared using plasma from phenylhydrazine-treated sheep. The cultures were incubated for 3 days in the conditions described above. Only those colonies containing more than eight cells were scored, and the mean number of CFU_e per femur ± SE was then determined.

Evaluation of results.—In treated mice and controls, single data points were examined on the same day by the same assay, to minimize possible day-to-day variations of the assays. In time-response experiments, the differences between data obtained in 4′-epi-DX- or DX-treated mice and between each of these groups and controls were evaluated by Student's t test. In the figures, the values of CFU_s, CFU_c, and CFU_e per femur were normalized to the untreated controls. The absolute numbers of HPC, bm cells, wbcs, and reticulocytes in controls are included in the legend of figure 1 and in table 1. The linear regressions between drug doses and survival fractions were fitted by the least squares method. Linear regression coefficients (r) and their significance were calculated. Comparison among sensitivities of each population assayed to each drug was performed by analysis of variance (F variance ratio and its significance) (25).

RESULTS

General Toxicity

In experiment B, the spontaneous death rate during the 10-day period of observation after treatment with three doses of 4.5 mg/kg was 3% in the DX-treated mice and 0 in the 4′-epi-DX-treated animals. No severe toxic signs were observed in surviving mice. During the 10 weeks of experiment C, spontaneous deaths were observed in 10% of the DX-treated mice and in 3% of the 4′-epi-DX group. Toxic signs were not evident in the survivors.

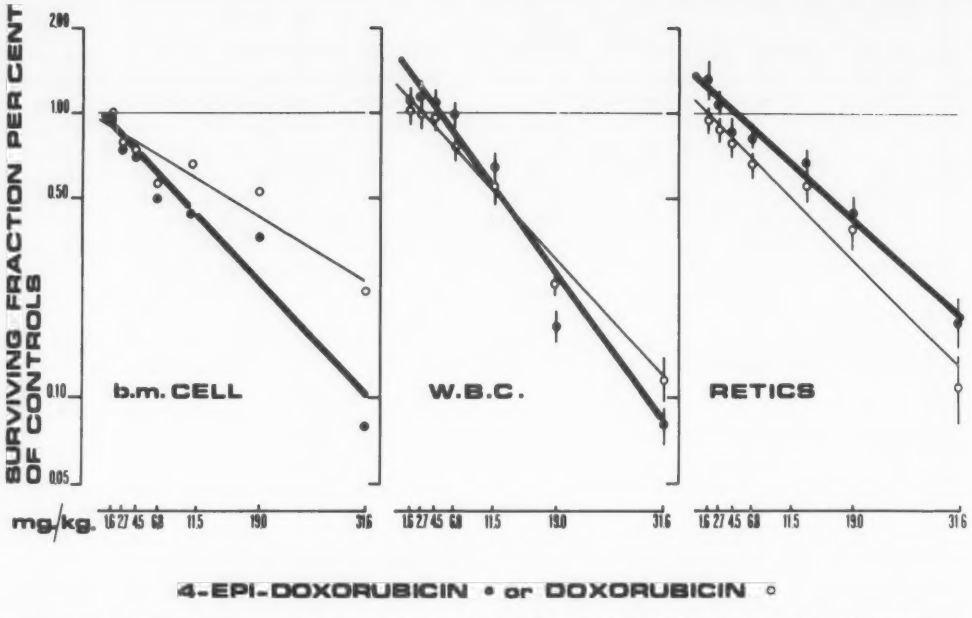

FIGURE 2.—Dose-survival curves of bm and peripheral blood cells 24 hrs after 4′-epi-DX and DX single injection. For each experimental point, vertical bars = mean ± SE. 4′-Epi-DX: bm cells, r = –0.967 ($P < 0.01$); wbc counts, r = 0.980 ($P < 0.01$); reticulocyte counts (retics), r = –0.986 ($P < 0.01$). DX: bm cells, r = –0.949 ($P < 0.01$); wbc counts, r = –0.994 ($P < 0.01$); reticulocyte counts, r = –0.986 ($P < 0.01$).

Dose-Survival Curves of Peripheral Blood Cells, Bone Marrow Cells, and HPC 24 Hours After 4′-Epi-DX Single Injection

Results are reported in figures 1 and 2. After 4′-epi-DX, within the dose range used, the survival curves for every cell population assayed were exponential (for each regression coefficient, $P < 0.01$). After comparison by analysis of variance it was seen that the slopes of the survival curves for CFU_s, CFU_c, CFU_e, wbcs, and reticulocytes showed a similar sensitivity ($P > 0.05$). On the other hand the dose-survival curve of bm cells indicated a higher sensitivity ($P < 0.01$). However, values obtained by calculation of the level at which the exponential line crosses the abscissa were 87%, 78%, and 66% of survival for CFU_s, CFU_c, and CFU_e, respectively, and 99%, 154%, and 129% for bm cells, wbcs, and reticulocytes, respectively. The lower extrapolation numbers for HPC may indicate that at low doses 4′-epi-DX has a greater impact on stem cells than on more mature cells. A comparison by analysis of variance of the dose-survival curves after 4′-epi-DX and after DX single doses showed similar slopes in the two groups ($P < 0.05$). However, with regard to HPC, the extrapolation numbers for DX (62%, 45%, and 44% of survival for CFU_s, CFU_c, and CFU_e, respectively) were lower than for 4′-epi-DX, while differences were much less marked for bm cells, wbcs, and reticulocytes (94%, 128%, and 111%, respectively) in DX-treated mice.

Moreover, considering single experimental points, there is a statistically significant difference (Student's t test: $P < 0.01$) between survival fractions of HPC of the two groups at the following doses: CFU_s—4.5, 6.8, and 19 mg/kg; CFU_c—4.5, 6.8, and 11.5 mg/kg; and CFU_e—2.7, 4.5, 6.8, 19.0, and 31.6 mg/kg. No statistically significant difference was detectable for the other parameters, except for wbcs at a dose of 6.8 mg/kg and reticulocytes at 1.6 mg/kg.

Effects on Peripheral Blood Cells, Bone Marrow Cells, and HPC of an Intermittent Treatment Schedule With 4′-Epi-DX

The survival of HPC, bm cells, wbcs, and reticulocytes as a function of time during the following intermittent administration of 4′-epi-DX is represented in figure 3 and reported in table 1 together with the sequence for mice treated with the same schedule and dose of DX. The pattern of CFU_s changes was characterized by an initial (Day 3) severe depletion followed by a prompt recovery (Day 6), overshooting the normal level. The administration of the third dose of the drug

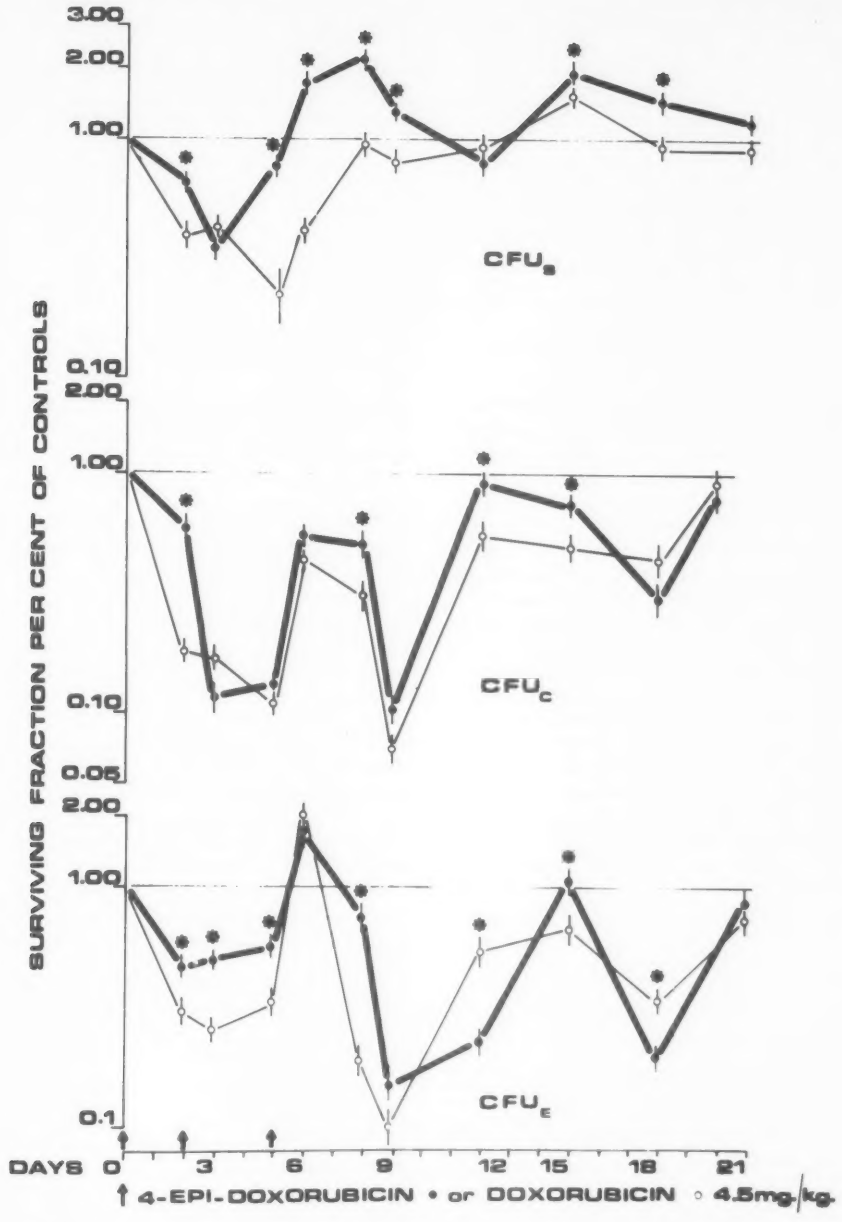

FIGURE 3.—Fractional survival of CFU as function of time during and after intermittent treatment schedule with DX and 4'-epi-DX. For each experimental point, vertical bars = mean ± SE. Values marked with asterisk are significantly different ($P < 0.05$) from corresponding values (○) of DX-treated animals.

TABLE 1.—Effects of an intermittent

	Values in untreated mice	Drug (4.5) mg/kg	2	3	5
VPRC (%)	44 ± 0.8	4′-Epi-DX	44 ± 0.9	43 ± 1.0	45 ± 0.3
		DX	41 ± 2.4	44 ± 1.2	45 ± 1.7
Wbc count ($\times 10^9$ cells/L)	5.0 ± 0.2	4′-Epi-DX	4.9 ± 0.3	3.8 ± 0.4	2.0 ± 0.1†,‡
		DX	5.5 ± 0.3	4.3 ± 0.7	4.0 ± 0.1†
Reticulocyte count (cells/100 rbcs)	2.7 ± 0.3	4′-Epi-DX	2.3 ± 0.2	2.4 ± 0.2	3.3 ± 0.1
		DX	2.2 ± 0.2	2.3 ± 0.2	3.0 ± 0.1
Bm cellularity (cells $\times 10^6$)	13.5	4′-Epi-DX	7.8	12.0	14.4
		DX	8.0	7.3	9.2

*Values = mean ± SE. VPRC = volume of packed rbcs.
†Values are significantly different ($P < 0.05$) from those found in untreated mice.
‡Values are significantly different ($P < 0.05$) from those found in DX-treated mice.

did not interfere with this recovery. The pattern of CFU_c and CFU_e changes in the first 6 days of observation was similar to that of CFU_s changes, but the initial recovery was transitory, and a second much more severe reduction of CFU_c and CFU_e took place. This was followed by a second recovery, a third drop, and finally, a return to normal values. The overall pattern was one of oscillations at and below the normal level. In DX-treated mice the drop of CFU_s was more rapid and their recovery slower. There was no initial phase of overshooting. The sequence of CFU_c and CFU_e changes was, however, similar to that observed in 4′-epi-DX-treated animals with minor, although statistically significant differences; this is evidence that DX also has a more marked effect on committed progenitors. In bm cellularity and peripheral blood cell counts (table 1), the sequences of changes during and following 4′-epi-DX or DX are similar with few, inconsistent statistically significant differences. At the end of the experimental period (Day 21), every blood parameter checked was normal again.

A schedule of intermittent treatment with 4′-epi-DX appeared to have less effect on HPC, and pluripotential stem cells in particular, than a similar schedule of DX. The effects of the two schedules on bm cellularity and on peripheral blood cell counts, however, were almost the same.

Effects on Peripheral Blood Cells, Bone Marrow Cells, and HPC of Prolonged Treatment With 4′-Epi-DX Twice a Week

As shown in table 2, the sequence of the changes of peripheral blood cell counts and of bm cellularity observed during prolonged 4′-epi-DX administration showed only minor or transitory differences, compared with those induced by DX. In both DX- and 4′-epi-DX-treated animals, the main feature was an almost constant reduction of the wbc counts, which reached a severe degree at Weeks 4, 9, and 10. This was accompanied by a slight but constant decrease of the volume of packed rbcs. The CFU_s content of the bm (fig 4) remained normal or reached values significantly above normal in both groups of mice, except for a mild reduction at Week 10 in 4′-epi-DX-treated mice and at Weeks 1 and 10 in DX-treated animals. In DX-treated mice the CFU_c and CFU_e

TABLE 2.—Effects of prolonged 4′-epi-DX

	Values in untreated mice	Drug (2.5 mg/kg)	1	2	3
VPRC (%)	44 ± 0.8	4′-Epi-DX	42 ± 0.6	39 ± 0.6†	34 ± 0.6†,‡
		DX	38 ± 0.9†	38 ± 0.8†	39 ± 0.4†
Wbc count ($\times 10^9$ cells/L)	5.0 ± 0.2	4′-Epi-DX	3.1 ± 0.3†	4.2 ± 0.9	3.4 ± 0.3†
		DX	3.0 ± 0.2†	4.8 ± 0.6	3.5 ± 0.1†
Reticulocyte count (cells/100 rbcs)	2.7 ± 0.3	4′-Epi-DX	3.5 ± 0.4	3.9 ± 0.2†,‡	3.1 ± 0.3‡
		DX	3.2 ± 0.4	2.4 ± 0.3†	3.4 ± 0.2
Bm cellularity (cells $\times 10^6$)	13.5	4′-Epi-DX	14.0	14.0	13.2
		DX	8.0	11.0	14.0

*Values = mean ± SE. VPRC = volume of packed rbcs.
†Values are significantly different ($P < 0.05$) from those found in untreated mice.
‡Values are significantly different ($P < 0.05$) from those found in DX-treated mice.

4′-epi-DX and DX schedule*

Treatment Day						
6	8	9	12	15	18	21
41 ± 0.6	40 ± 0.9	41 ± 1.7	29 ± 1.9†	34 ± 3.0	38 ± 1.2	46 ± 1.6
42 ± 0.6	40 ± 0.6	41 ± 1.2	33 ± 2.3†	39 ± 2.7	40 ± 0.9	40 ± 2.1
4.3 ± 0.1	7.5 ± 0.3†,‡	3.7 ± 0.6	5.3 ± 0.3	2.4 ± 0.2†	4.3 ± 0.4	6.3 ± 0.4
5.4 ± 0.8	3.4 ± 0.2†	3.9 ± 0.2†	3.4 ± 0.4†	3.8 ± 0.2	2.8 ± 0.3†	5.3 ± 0.4
4.2 ± 0.3†	3.3 ± 0.1	2.7 ± 0.2	4.3 ± 0.2†,‡	6.0 ± 0.4†,‡	2.5 ± 0.2‡	3.0 ± 0.3
3.4 ± 0.2	3.2 ± 0.1	3.1 ± 0.2	2.0 ± 0.1	4.1 ± 0.3†	1.4 ± 0.2†	2.6 ± 0.2
12.6	23.0	9.0	7.2	19.2	8.6	16.7
11.2	10.0	6.6	10.1	23.1	7.8	20.1

content of the bm was just below or well below the normal value, for most of the observation period. Steep falls alternated with abortive or transitory recoveries. At Week 10 of treatment the bm content of CFU_c was at its lowest level. In 4′-epi-DX-treated mice, the general trends of CFU_c and CFU_e were similar, but their levels were generally higher than in the DX group. Five of ten CFU_c determinations were in or above the normal range. However, at Week 10 of treatment, in both groups of animals, there was a severe depletion of committed progenitors, which contrasted with the nearly normal endowment of the pluripotential stem cells.

DISCUSSION

The antitumor activity of 4′-epi-DX has been evaluated on experimental tumors and more recently also in clinical trials. In mice with leukemias L1210 or P388, Gross' leukemia, solid Sarcoma 180, or transplanted early and advanced mammary carcinoma (7–9), the antitumor effect of 4′-epi-DX was comparable to that of DX. The analog, however, was better tolerated. Evaluating new anthracycline compounds by the human tumor stem cell assay, Salmon et al (10) have shown that 4′-epi-DX and DX have almost the same potency of inhibition (46%–47%). In humans, too, the anticancer values of 4′-epi-DX and DX were substantially comparable, the new analog having the advantage of a lower incidence of marrow suppression and of other side effects (11). In another study (12), 4′-epi-DX showed a less marked hematologic toxicity and a more rapid elimination than DX. Preliminary results of a phase II trial of 4′-epi-DX for advanced solid tumors (13) indicated that overall efficacy of the drug is similar to that of DX in breast cancer. Again, the new analog appeared to be less toxic than the parent drug. These results tend to suggest that 4′-epi-DX is a valid alternative to DX. This is further supported by the data showing that, in mice, 4′-epi-DX seems to have a lower impact on HPC than DX. When the toxic effect is assessed 24 hours after a single dose of either drug, the slopes of the survival curves for the three types of HPC assayed in 4′-epi-DX-treated mice are statistically similar to those for DX-treated mice. The lower extrapolation levels at which the exponential lines cross the abscissa in 4′-epi-DX-treated animals and the statistically significant differences be-

and DX treatment twice a week*

Treatment Week						
4	5	6	7	8	9	10
34 ± 0.6†,‡	31 ± 1.4†,‡	37 ± 0.6†	39 ± 0.6†	35 ± 0.3†	34 ± 0.6†	30 ± 0.3†
39 ± 0.6	38 ± 0.4†	37 ± 0.6†	37 ± 0.6†	33 ± 2.0†	34 ± 1.4†	25 ± 0.3†
2.6 ± 0.3†	3.4 ± 0.5†	3.5 ± 0.2†	3.4 ± 0.5†	3.6 ± 0.4†	2.8 ± 0.4†	2.6 ± 0.3
2.4 ± 0.3†	3.9 ± 0.6†	4.5 ± 0.2	3.2 ± 0.3†	3.4 ± 0.3†	2.5 ± 0.2†	3.0 ± 0.6
3.6 ± 0.4†	4.4 ± 0.6†	3.4 ± 0.3	3.2 ± 0.2	2.5 ± 0.4‡	2.5 ± 0.4	1.3 ± 0.1†,‡
3.1 ± 0.6	4.2 ± 0.2†	3.7 ± 0.3†	3.3 ± 0.2	3.3 ± 0.2	2.4 ± 0.3	2.0 ± 0.1†
9.4	12.8	15.4	12.8	9.6	15.1	5.0
10.0	10.0	11.6	10.4	6.7	14.3	5.1

SURVIVING FRACTION PER CENT OF CONTROLS
3.00
2.00
1.00
0.10
0.01
CFU$_S$
CFU$_C$
CFU$_E$
WEEKS 0 1 2 3 4 5 6 7 8 9 10 0 1 2 3 4 5 6 7 8 9 10 0 1 2 3 4 5 6 7 8 9 10
4-EPI-DOXORUBICIN • or DOXORUBICIN ○ 2.5mg/kg

FIGURE 4.—Fractional survival of CFU as function of time during treatment with DX and 4'-epi-DX twice/wk. For each experimental point, vertical bars = mean ± SE. Values marked with asterisk are significantly different ($P < 0.05$) from corresponding values (○) of DX-treated animals.

tween survival fractions at most single experimental points, however, indicate that the effect of 4′-epi-DX on HPC is less toxic. On the contrary, there are no or only minor differences in bm cellularity and wbc and reticulocyte counts. Also, when the drugs were administered on an intermittent schedule, the pluripotential stem cells and, to a lesser degree, the committed stem cells seemed to be less affected by 4′-epi-DX than by DX. Finally, when the drugs were given for a relatively long period, the difference between 4′-epi-DX and its parent compound was particularly evident as far as the committed stem cells were concerned, while the milder involvement of the CFU_s prevented the detection of any possible differences of sensitivity to the two drugs. Any discussion of the difference between the effects of DX and its derivative 4′-epi-DX can only be a matter of conjecture. The survival-dose curves of precursor cells for both drugs are exponential, indicating that both DX and 4′-epi-DX are cycle phase nonspecific agents. Moreover, CFU_s are primarily a resting population, whereas committed progenitors (CFU_c and CFU_e) include a much higher proliferating fraction. Differences in the effects of DX and 4′-epi-DX were observed, however, for all three types of cells. These considerations seem to rule out the possibility that the kinetics of the target cells are conclusive in accounting for these differences.

4′-Epi-DX has a distribution in the tissues similar to that of DX (7), but it is retained less in the organs. If one assumes that recruitment of resting cells begins in the 24 hours following drug administration, the cell entering in proliferation could be differently affected if exposed to a different tissue level of the cytotoxic drug. Thus, the prompter reduction of tissue level of 4′-epi-DX compared with DX could lead to a lowering of the toxicity on recruited cells and account for what seems to be a less marked effect of 4′-epi-DX on HPC.

It is also possible that more subtle biologic differences between the two drugs are responsible for the differences in toxic effects on the very immature hematopoietic stem cells. Di Marco et al (26) showed that differences in polarity and in pKa values between DX and 4′-epi-DX are related to their different antitumor activity and toxicity in leukemia P388-bearing mice and to their uptake by leukemia L1210 cells. The finding that after 4′-epi-DX the dose-survival curve for bm cellularity shows a greater decline than after DX is very intriguing, particularly if one considers the concomitant absence of consistent differences in peripheral blood parameters. No ready explanation seems available at the moment, and more experiments will be necessary to clarify this result.

When cytotoxic drugs are administered for a relatively long time, a very intricate interplay of opposing factors must be taken into account. Among these, the cumulative toxicity of repeated doses of drugs, on the one hand, and the compensating mechanisms operating inside the target populations, on the other hand, are of primary importance. This complexity of changes taking place both inside and outside the hematopoietic system does not allow a valid analysis of the sequence of progenitor cell variations observed during and following intermittent and prolonged administration of the anthracyclines studied. However, the general trend of results obtained open the door to some significant considerations.

In the short schedule of intermittent treatment (experiment B), 4′-epi-DX shows a lower toxic effect than DX on CFU_s and also partially on CFU_c and CFU_e, without consistent differences in blood parameters. This might suggest a similar effect of both drugs on more advanced stages of myelopoiesis and, consequently, a similar immediate hematotoxic pattern.

In 4′-epi-DX-treated mice, however, the self-renewing capacity of the hematopoietic system is partially spared, and these animals have a greater chance of full recovery from the drug insult. Thus, the long-term results obtained with this short schedule of treatment, which assures a lower general toxicity and greater antileukemic activity of DX, both in normal and tumor-bearing animals (1), could be further improved by substituting 4′-epi-DX for DX.

In the experiment of long-term toxicity (10 weeks), both drugs caused a similar reduction of wbc count, but 4′-epi-DX had a less marked effect on committed stem cells than DX. This again is evidence of similar action of the two drugs in the more advanced stages of myelopoiesis and of a lesser toxicity of 4′-epi-DX in the earlier phases of hematopoiesis. Thus, prolonged administration of 4′-epi-DX, while causing an apparent hematologic toxicity of the same degree as DX, may in fact have a lesser effect on the progenitor compartments, thus allowing longer treatment. Results obtained after 10 weeks of treatment may, however, indicate that, in the long run, the two drugs reach an equal degree of toxicity on stem cells. This may suggest that the lesser toxicity of 4′-epi-DX is exploitable only with a short schedule of treatment.

Previous experience (27–29) indicates that there are discrepancies between the effects of cytotoxic drugs on morphologically recognizable hematopoietic cells and on colony-forming precursors. In this study, most of the results show that there are no great differences between the effects of DX and 4′-epi-DX on peripheral blood cell counts or bm cellularity. However, the difference becomes evident at the HPC level. It appears, therefore, that HPC assays, when possible, would constitute a much more sensitive means for assessing the degree of drug myelotoxicity in man.

REFERENCES

1. Casazza AM. Preclinical studies for the evaluation of new anthracycline analogs. Chemiother Oncol 4:310–326, 1978.
2. Deckers C, Mace F, Deckers-Passen C, et al. The antitumor activity of adriamycin-DNA complex in experimental tumors. *In* Molecular Base of Malignancy (Deutsch D, et al, eds). Stuttgart, Thieme, 1976, pp 254–262.
3. Alberts DS, and Van Dalen Vetters T. Rubidazone vs adriamycin: an evaluation of their differential toxicity in the spleen colony assay system. Br J Cancer 32:64–69, 1976.
4. Parker LM, Hirst M, and Israel M. *N*-trifluoroacetyladriamycin-14-valerate: additional mouse antitumor and toxicity studies. Cancer Treat Rep 62:119–127, 1978.
5. Arcamone F, Di Marco A, and Casazza AM. Chemistry and pharmacology of new antitumor anthracycline. *In* Advances in Cancer Chemotherapy (Vruezawa H, et al, eds). Tokyo, Japan Science Society Press and Baltimore, University Park Press, 1978, pp 297–312.
6. Bhuyan BK. Cell-kill kinetics and cell-cycle effects of nogalamycin and 7-*O*-methylnogarol. Proceedings Congressi Farmitalia, 1978, p 47.
7. Casazza AM, Di Marco A, Bertazzoli C, et al. Antitumor activity, toxicity, and pharmacological properties of 4′-epiadriamycin. Curr Chemother 11:1257–1260, 1978.
8. Casazza AM, Di Marco A, Bonadonna G, et al. Effects of modifications in position 4 of chromophore in position 4′ of the aminosugar on the antitumor activity and toxicity of daunorubicin and doxorubicin. *In* Anthracyclines. Current Status and New Developments (Crooke ST, and Reich SD, eds). New York, Academic Press, 1980, pp 403–430.
9. Arcamone F. Doxorubicin. *In* Cancer Antibiotics. New York, Academic Press, 1981.
10. Salmon SE, Liu RM, and Casazza AM. Evaluation of new anthracycline analogs with the human tumor stem cell assay. Cancer Chemother Pharmacol 6:103–109, 1981.
11. Bonfante V, Bonadonna G, Villani F, et al. Preliminary phase I study of 4′-epi-Adriamycin. Cancer Treat Rep 63:915–918, 1979.
12. Natale N, Brambilla M, Luchini S, et al. (Abstr) 4′-Epi-doxorubicin and doxorubicin: toxicity and pharmacokinetics in cancer patients. *In* Proceedings of the 12th International Congress of Chemotherapy, Florence, Italy, July 19–24, 1981, p 114.
13. Hurteloup P. (Abstr) A phase II trial of 4′-epi-adriamycine (4′ EA) for advanced solid tumors. Preliminary results. *In* Proceedings of the 12th International Congress of Chemotherapy, Florence, Italy, July 19–24, 1981, p 115.
14. Lohrmann HP, and Schreml W. Cytotoxic drugs and the granulopoietic system. Berlin/Heidelberg, Springer Verlag, 1982.
15. Marsh JC. Comparison of the sensitivities of human, canine and murine hematopoietic precursor cells to adriamycin and *N*-trifluoroacetyladriamycin-14-valerate. Cancer Res 39:360–364, 1979.
16. Massa G, Bogliolo G, D'Amore F, et al. Hemopoietic precursor cells in mice treated with 4-demethoxydaunorubicin and doxorubicin. JNCI 68:971–976, 1982.
17. Buick N, and McCulloch EA. A colony assay for human leukemic blast cells used to measure anthracycline sensitivity in culture. Proceedings Congressi Farmitalia, 1978, p 23.
18. Razek A, Valeriote F, and Vietti T. Survival of haematopoietic and leukemic colony-forming cells in vivo following the administration of daunorubicin or adriamycin. Cancer Res 32:1496–1500, 1972.
19. Di Marco A, Casazza AM, Gambetta R, et al. Relationship between activity and amino sugar stereochemistry of daunorubicin and adriamycin derivates. Cancer Res 36:1962–1966, 1976.
20. Di Marco A, Zunino F, and Casazza AM. Comparison of biochemical and biological methods in the evaluation of new anthracycline drugs. Antibiot Chemother 23:12–20, 1978.
21. Dacie JV, and Lewis SM. Practical Haematology. Edinburgh, Churchill-Livingstone, 1975.
22. Bradley TR, and Metcalf D. The growth of mouse bone marrow cells in vitro. Aust J Exp Biol Med Sci 44:287–300, 1966.
23. Till JE, and McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 14:213–222, 1961.
24. Iscove NN, Sieber F, and Winterhalter KH. Erythroid colony formation in cultures of mouse and human bone marrow: analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on Agarose-Concanavaline A. J Cell Physiol 83:305–320, 1973.
25. Bliss CI. Statistics in Biology. New York, McGraw-Hill, 1970.
26. Di Marco A, Casazza AM, Desdia T, et al. Changes of activity of daunorubicin, adriamycin and stereoisomers following the introduction or removal of hydroxyl groups in the amino sugar. Chem Biol Interact 19:291–302, 1977.
27. Breivik H. Response of multipotent (CFU) and granulocyte (diffusion chamber assay) progenitor cells and differentiating cells of murine haematopoietic tissue to a perturbation of the steady state. J Cell Physiol 79:171, 1971.
28. Jenkins VK, Costanzi JJ, and Ellis HN. Effects of single and combined chemotherapeutic agents on hemopoietic stem cells in mice. Am J Haematol 1:79, 1976.
29. Pannacciulli IM, Massa GG, Saviane AG, et al. The effects of chronic administration of cyclophosphamide on haemopoietic stem cells. Scand J Haematol 19:217, 1977.

Cytotoxic and Cell Kinetic Effects of 3,4,5-Trihydroxybenzohydroxamic Acid (VF 122) in Hepatoma 3924A Cells[1,2]

Jasna Ban,[3,4] Editi Olah,[3,5] and George Weber[3,*]

3,4,5-Trihydroxybenzohydroxamic acid (VF 122), an inhibitor of ribonucleotide reductase, killed rat hepatoma 3924A cells in tissue culture after 7 days of incubation. A concentration of 15 μM caused 50% inhibition of colony-forming ability (IC_{50}). Under the same conditions, hydroxyurea, also an inhibitor of ribonucleotide reductase, had an IC_{50} of 52 μM. Treatment for 1 hr with VF 122 of exponentially growing culture resulted in a biphasic exponential dose-response curve (D_0 = 0.5 mM, IC_{50} = 0.75 mM, and D_0 = 1.8 mM, IP = 0.8 mM). In plateau-phase cells, a threshold exponential curve was obtained (D_0 = 2.2 mM, D_q = 1.7 mM, and IC_{50} = 2.7 mM). Exponentially growing hepatoma 3924A cells were more sensitive to VF 122 than were plateau-phase cultures. In contrast, hydroxyurea killed only exponentially growing 3924A hepatoma cells, exhibiting an exponential plateau dose-response curve without achieving an IC_{50} value at concentrations from 1 to 200 mM. In synchronized cultures, VF 122 (1 mM for 1 hr) was toxic for cells in mid and late G_1 phase, in early and mid S phase, and, to a lesser degree, in G_2 phase. Hydroxyurea (10 mM for 1 hr) killed cells only in S phase. Proliferating and resting hepatoma 3924A cells recovered from sublethal and potentially lethal damage induced by VF 122. [Cancer Treat Rep 66:2071–2080, 1982]

Until recently, hydroxyurea (HU) was the main drug in clinical use with a primary target of ribonucleotide reductase (1–4). This neoplastic progression-linked key enzyme (5–7) catalyzes the reductive conversion of ribonucleotide to deoxyribonucleotide, leading to DNA synthesis and cell replication (8). Some newly synthesized derivatives of benzohydroxamic acid proved to be better inhibitors of the reductase activity and tumor growth than HU. Among them was 3,4,5-trihydroxybenzohydroxamic acid (VF 122), a hydroxy derivative of benzohydroxamic acid. VF 122 inhibited ribonucleotide reductase in vitro (10 μM caused 50% inhibition of colony-forming ability [IC_{50}]) and increased survival time of leukemic mice by 78% (9,10). A structure-activity relationship study indicated that the ring substituents play an important role in the inhibitory potency of hydroxamic acid (11). It was determined that 3,4-dihydroxybenzohydroxamic acid was the most potent derivative in this series (9,10), but recent work in this laboratory showed that according to IC_{50} values of colony-forming studies, VF 122 was five times stronger as a killing agent for rat hepatoma 3924A cells (J. Ban and G. Weber, unpublished observation).

The action of VF 122 was investigated to elucidate the mechanism of cytotoxicity on proliferating and resting rat hepatoma 3924A cells in tissue culture as measured by colony-forming ability. The lethality, as a function of VF 122 concentration and time exposure as well as of cell cycle-dependent toxicity, was studied and compared with results obtained with HU. In addition, the response of proliferating and resting tumor cells to drug-induced damage was examined.

[1]Received June 1, 1982; revised Sept 9, 1982; accepted Sept 28, 1982.

[2]Supported by Public Health Service grants CA-13526 and CA-05034 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Laboratory for Experimental Oncology, Indiana University School of Medicine, Indianapolis.

[4]*Present address:* Central Institute for Tumors and Allied Diseases, Zagreb, Yugoslavia.

[5]*Present address:* Research Institute of Oncopathology, Budapest, Hungary.

**Reprint requests to:* George Weber, MD, Laboratory for Experimental Oncology, Indiana University School of Medicine, 1100 W Michigan St, Indianapolis, IN 46223.

MATERIALS AND METHODS

Cell culture.—Hepatoma 3924A cells were grown in monolayer cultures in McCoy's 5A medium with 10% fetal calf serum (Grand Island Biological Co, Grand Island, NY) and were incubated in a 5% CO_2:95% air atmosphere. To keep the cells in logarithmic growth, they were subcultured three times a week. For experiments on exponentially growing populations, 5×10^5 hepatoma 3924A cells were seeded into plastic flasks (surface area, 25 cm^2) and incubated for 48 hrs. For studies on plateau-phase cells, cultures were incubated for 96 hrs without changing the medium. Experimental methods, definitions, and cytogenetic characteristics of plateau and exponential phases have been reported elsewhere (12).

Synchronization technique.—Synchronized cell populations were used to study the cell cycle phase specificity of the drug. Synchrony was achieved by mitotic selection (13,14). The mitotic index was 90% as determined by counting metaphases after aceto-orcein staining in a hemocytometer. The length of the phases of the cell cycle was determined as reported previously (12). Current results indicated that the duration of each phase was as follows: G_1, 7 hrs; S, 4.5 hrs; and G_2, 2.5 hrs. The average cell cycle time was 14 hrs (15).

Dose-response experiments.—Exponential- or plateau-phase cells were treated with different concentrations of drugs. Cultures were exposed to VF 122 or HU for 1 hr, the drug was then removed, flasks were rinsed three times with phosphate-buffered saline (PBS) containing (in g/L) KCl (0.20), KH_2PO_4 (0.20), NaCl (8.00), and $Na_2HPO_4 \cdot 7H_2O$ (2.16). The cells were then trypsinized, counted with an electronic counter (Coulter Electronics, Inc, Hialeah, FL), and seeded into culture flasks (500 cells/25 cm^2). The pH was adjusted with 5% CO_2 in air. After 7 days of incubation, the medium was removed and the cells were stained with a saturated solution of crystal violet in 0.85% sodium chloride (12). Colonies containing > 50 cells were counted and results were expressed as a percent of colonies of untreated cells. To avoid growth stimulation, plateau-phase cells were treated by adding the drugs (prepared in PBS) to the exhausted medium for 1 hr; cells were then trypsinized and plated as described above. Long-term experiments were performed with proliferating cultures. Cells were seeded (500/flask) and exposed to the drug for 7 days, as reported elsewhere (15). Colonies were stained and counted as above. The plating efficiencies of untreated control cultures of exponentially growing cells, plateau-phase cells, and mitotically selected cells were 76%, 72%, and 50%, respectively. Mean, standard error, and statistical evaluation by the t test were calculated for each dose or time point. Differences between mean values yielding a probability of < 5% were considered significant.

Recovery from sublethal damage.—As a test for recovery from sublethal damage, fractionated doses were used. The treatment was fractionated using the total dose concept of drug concentration and duration of treatment (C × T) (16). Exponential- or plateau-phase cells received the total dose in two equal sequential exposures. All cells received one-half of the total dose of VF 122 at 0 hr. At the end of the first treatment (30 mins), one group of cells was immediately given the same dose, thus allowing no time between treatments for recovery. The other groups of cells were washed and reincubated in fresh or in depleted plateau-phase medium, depending on the cell growth phase; they were exposed for 30 mins to the second half of the total dose 1–6 hrs later. All cells were plated for colony formation immediately after exposure to the second dose of the drug. Controls obtained at regular intervals consisted of cells given either one-half dose or the total dose (for details *see* figs 5 and 6).

Recovery from potentially lethal damage.—Exponential- or plateau-phase cells were treated with different concentrations of VF 122 for 30 or 60 mins. Following treatment, cells were plated immediately for colony formation or were washed three times with PBS and incubated at 37°C in depleted plateau-phase culture medium (96 hrs old) for 2, 4, or 6 hrs before being plated for colony formation.

Drugs.—VF 122 was obtained from Dr. H. L. Elford, College of William and Mary, Newport News, VA. HU was purchased from Sigma Chemical Co, St. Louis. Drugs were stored under refrigeration and prepared with sterile PBS before use.

RESULTS AND DISCUSSION

Dose-Response Curves of VF 122 and Hepatoma 3924A Cells

The threshold-exponential dose-response survival curve of proliferating cells exposed to VF 122 for 7 days yielded an IC_{50} of 15 μM (fig 1). The mean lethal dose (D_0, the concentration required to reduce survival by 63% on the exponential part of the survival curve) was 12 μM, and the quasi-threshold dose [D_q, equal to the intercept with the abscissa of the exponential part of the survival curve (17)] was 6 μM. A dose-response curve for rat hepatoma 3924A cells exposed to HU for 7 days is presented in figure 1 for comparison. A threshold-exponential type curve with a wide shoulder yielded an IC_{50} of 52 μM, a D_0 of 46 μM, and a D_q of 43 μM. According to IC_{50} values, VF 122 was 3.5 times more active than HU after long-term treatment.

Study of Growth Phase Sensitivity to VF 122

To determine whether there was a growth phase-dependent sensitivity to VF 122, exponentially growing

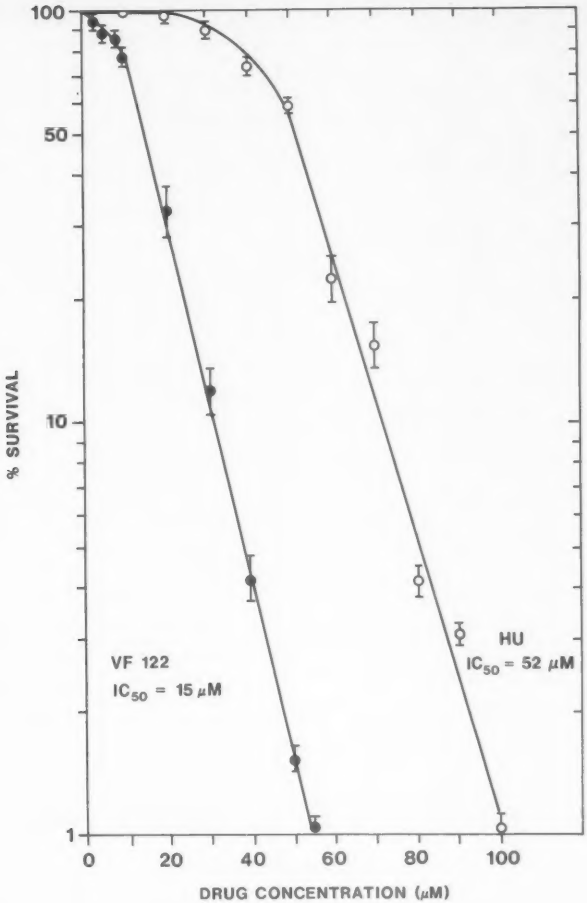

FIGURE 1.—Survival of proliferating hepatoma 3924A cells exposed to VF 122 or HU for 7 days. Cells were seeded (500/flask), supplemented with drugs, and incubated for 7 days. Colonies were counted, and counts were expressed as % of colonies of untreated cells. Each point is the average of triplicate samples of 3 experiments.

and plateau-phase cells were treated with the drug for 1 hr (fig 2). The treatment of exponentially growing cells resulted in a biphasic exponential dose-response curve with a D_0 of 0.5 mM for the initial part of the curve (IC_{50} = 0.75 mM) and a D_0 of 1.8 mM for the second part of the curve. The inflection point (IP), the concentration that eradicates the most sensitive cells from the population (17), was 0.8 mM. Plateau-phase cells were characterized by a threshold exponential curve with a moderately large shoulder region and exponentially decreased survival as a function of increasing concentrations (D_0 = 2.2 mM, D_q = 1.7 mM, and IC_{50} = 2.7 mM). Exponentially growing cells were more sensitive to VF 122 than plateau-phase cultures. This difference diminished when higher drug concentrations ($\geq$ 6 mM) were used (data not shown). These observations indicated that VF 122 could be a cycle-specific agent according to the kinetic classification of antitumor agents (18,19).

Since VF 122 revealed significant lethal effect against exponential- and plateau-phase cells, the data suggested that the drug might be effective not only for rapidly growing tumors but also for slowly growing solid tumors containing large fractions of resting cells. In

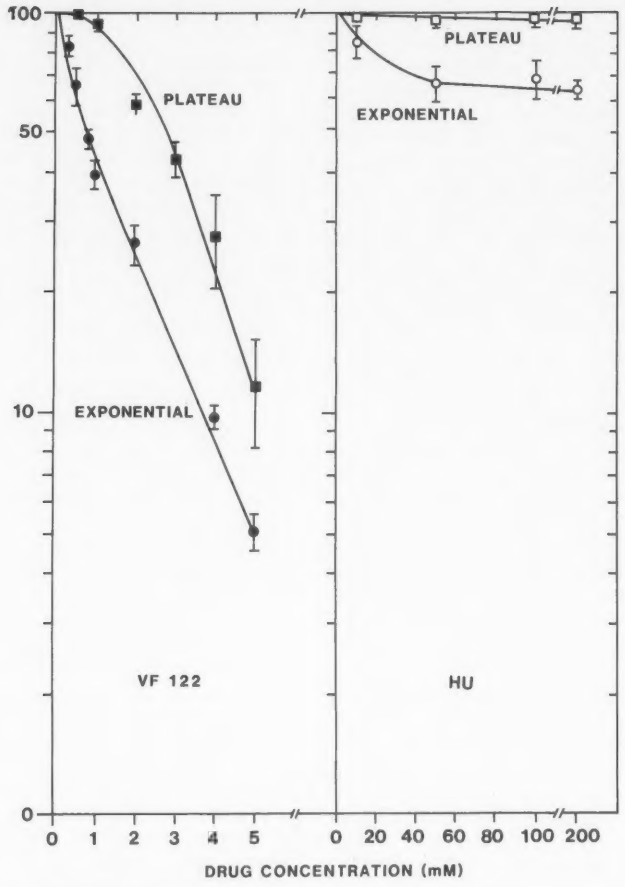

FIGURE 2.—Survival of hepatoma 3924A cells in exponential and plateau phases exposed to increasing concentrations of VF 122 and HU. Cells were treated for 1 hr, seeded, and counted after 7 days of incubation. Results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

contrast, HU did not kill the plateau-phase hepatoma 3924A cells (fig 2). Exponentially growing cells showed an exponential plateau dose-response survival curve that was characterized by an initial exponential decrease in survival (D_0 = 80 mM). A concentration of 50 mM killed 35% of the proliferating cells and then the surviving fraction leveled off, with no increased killing at doses as high as 200 mM. These results agreed with earlier observations on the action of HU on other cell lines (20–24).

Action of VF 122 in the Cell Cycle

It was reported previously (15,25) that cycle-specific agents produce significant fluctuations in age responses of synchronized cells; thus, it seemed desirable to compare the sensitivity of synchronized cells in different phases of the cell cycle (12–14). The position of cells in the cell cycle time was determined by pulse-labeling autoradiography (^{3}H-thymidine, 1.0 μCi/ml; specific activity, 2 Ci/mmol). Synchronized cultures were treated at regular intervals for 1 hr with 0.25 and 1 mM VF 122. This treatment killed 10% and 50%, respectively, of the cells in the asynchronous cell population, as determined by colony counts.

A concentration of 0.25 mM killed the cells chiefly in late G_1 and early S phase. The toxicity was expressed to a lesser degree in the early G_1 and late S phases.

There was only slight killing action on cells in other phases of the cycle (fig 3). The higher concentration of VF 122 (1 mM) was toxic also in mid G_1, mid S, and G_2 phases. Cells in the M phase were virtually insensitive to VF 122. The cells in G_1–S transition were five times more sensitive than those in G_2 phase. The greater sensitivity of cells in the G_1–S transition region could be explained by different sensitivity of the cell populations to the drug, as shown in figure 2, where the dose-survival curve of exponential cells had two different slopes. Inhibition of cell growth in S phase may also be attributed to an action of VF 122 on DNA synthesis, as measured in HeLa cells (H. L. Elford, personal communication).

HU (10 mM for 1 hr) showed clear-cut phase-specific toxicity, killing cells in S phase only (survival was 70%; fig 3). This agreed with findings in other cell lines (24,26).

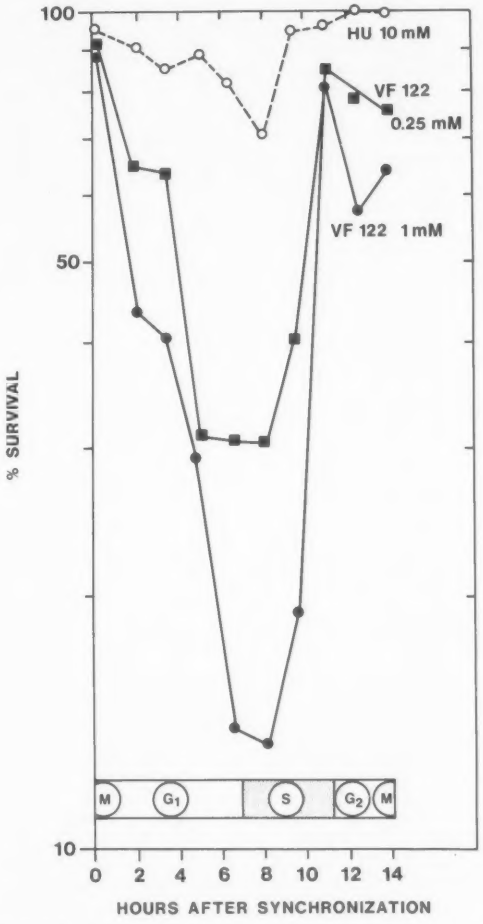

FIGURE 3.—Sensitivity of synchronized hepatoma 3924A cells to VF 122 (0.25 and 1 mM) and HU (10 mM). Synchronized cells (1000/flask) were exposed to drug at regular intervals after mitotic selection. Drug was removed after 1 hr, cells were washed with PBS, and fresh medium was added. Synchronized, untreated controls were also plated for each time point. Results were expressed as % of colonies of untreated cells and were evaluated as in fig 1. Each point is the average of triplicate samples of 2 experiments.

Stability of Biologic Activity of VF 122

It was important to determine the stability of VF 122 in cell culture because of the length of some of the experiments. Exponentially growing cells were exposed to 0.5 and 1 mM VF 122 for increasing periods of time. Biphasic time-dependent survival curves are presented in figure 4.

Here again, as in the dose-survival curve of proliferating cells (fig 2), two different slopes are evident. The final slopes appearing within 30–60 mins after VF 122 treatment manifested different time-dependent action of the drug, depending also on the concentration.

To examine the biologic stability of VF 122 in different solutions, the drug was dissolved and preincubated at 37°C in complete medium with serum and in PBS for different periods (up to 48 hrs). The drug solutions were then added to proliferating cells, cultures were incubated for 7 days, and plating efficiencies were compared. Since no difference in the cell survival was found, it was assumed that the drug was stable under these conditions (data not shown).

Response of Exponential- and Plateau-Phase Cells to Sublethal Damage

The shape of the dose-response survival curve of plateau-phase cells suggested their capacity to accumulate some damage without lethality, whereas no shoulder region was observed for the dose-response curve of proliferating cells. To test the recovery from sublethal damage (SLD), the treatments were fractionated using the integral dose concept (16), and fractions of the total dose, given sequentially, were used to treat proliferating and resting cells.

Survival of exponential cells that received two doses of 1 mM VF 122 for 30 mins, separated by 0, 1, 2, 4, 6, or 8 hrs, is shown in figure 5. The theoretical value for survival after two treatments was defined as the survival of cells that completed repair of SLD from the first dose, before the second dose was given (16). Therefore, the theoretical value for survival (S) after two treatments is $(\%S)_1 \times (\%S)_2/100$. If the surviving cells have complete repair of SLD from the first dose they should survive a second dose by the same fraction. The survival of exponentially growing hepatoma 3924A cells treated with a single dose of VF 122 (1 mM for 30 mins)

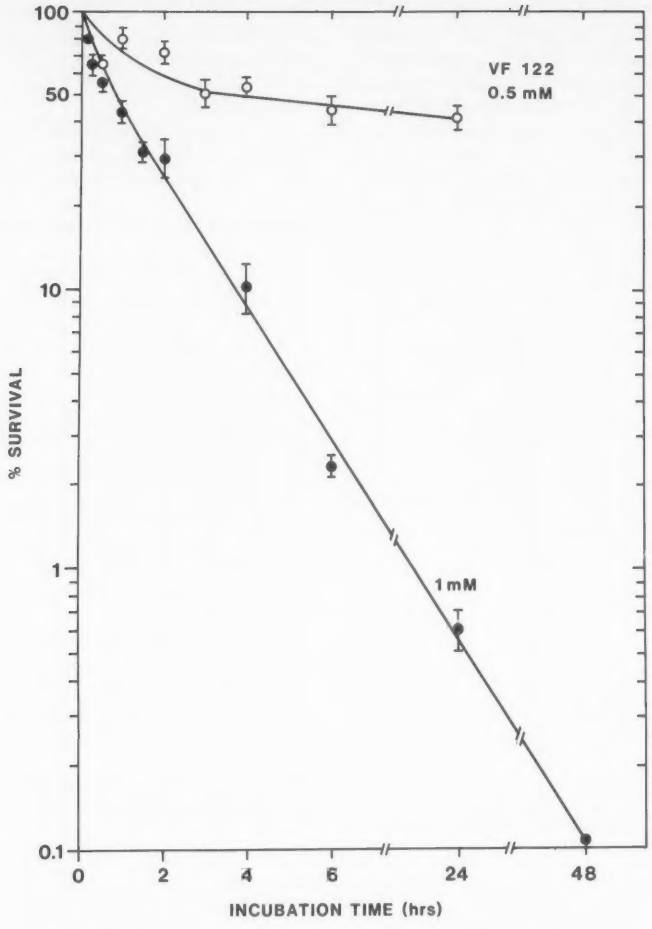

FIGURE 4.—Survival of proliferating hepatoma 3924A cells exposed to VF 122 as a function of time. Exponentially growing cells were treated with 0.5 or 1 mM VF 122 and then seeded for survival at regular intervals. Results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

was 57%; therefore, the theoretical value for survival was 32%. Figure 5 shows that recovery was observed even after 2 hrs; the 37% survival at this time point was nearly the same as the theoretical value. With an interval of 6 hrs between the doses, survival was 60%, which was 1.8 times higher than the theoretical value. In fact, this survival was nearly equal to that determined at zero time for the cells treated with one-half of the total dose (1 mM for 30 mins); it was also higher than survival, determined at zero time, of the cells that received the total dose at one time (2 mM for 30 mins or 1 mM for 60 mins).

Plateau-phase cells were also treated with fractionated doses. However, higher concentrations were given because of the lower sensitivity of these cells to VF 122. Between the two doses, cells were maintained in the medium from plateau-phase cultures to avoid growth stimulation. Figure 6 shows that plateau-phase cells recovered from SLD induced by VF 122 (3 mM for 30 mins, spaced in time). The survival of cells that received fractionated doses after 6 hrs of incubation was 1.8 times higher than the theoretical survival (30%), indicating

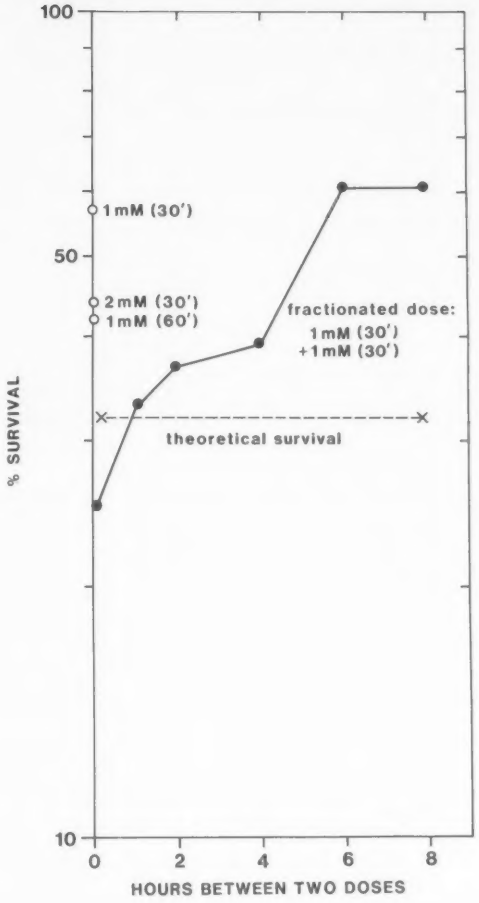

FIGURE 5.—Survival of exponentially growing hepatoma 3924A cells after total (2 mM for 30 mins or 1 mM for 60 mins), $^1/_2$ of total (1 mM for 30 mins), and fractionated-dose treatment with VF 122 (●). Cells that received fractionated doses were exposed to a single dose of 1 mM VF 122 for 30 mins at 0 time. 1 group of cells (0 time point) was treated immediately with same dose, with no time interval. Other groups of cells were washed with PBS and reincubated in fresh medium. At times indicated by points, cells were given same additional dose (1 mM for 30 mins) and were plated immediately for colony formation. Colonies were counted and results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

that resting cells also recovered from VF 122-induced damage.

Response of Exponential- and Plateau-Phase Cells to Potentially Lethal Damage

Since recovery from potentially lethal damage (PLD) could contribute to SLD recovery during the experiments designed to test for SLD, it was relevant to determine the response to PLD. The recovery from PLD was defined as the increase in survival of cells that were exposed to suboptimal growth conditions after a single drug treatment (16,27,28). Exponentially growing hepatoma 3924A cells were treated with a single dose of 1 or 2 mM VF 122 for 60 mins and were plated immediately for colony counts (survival rates were 42% and 25%, respectively). Another set of samples was given the

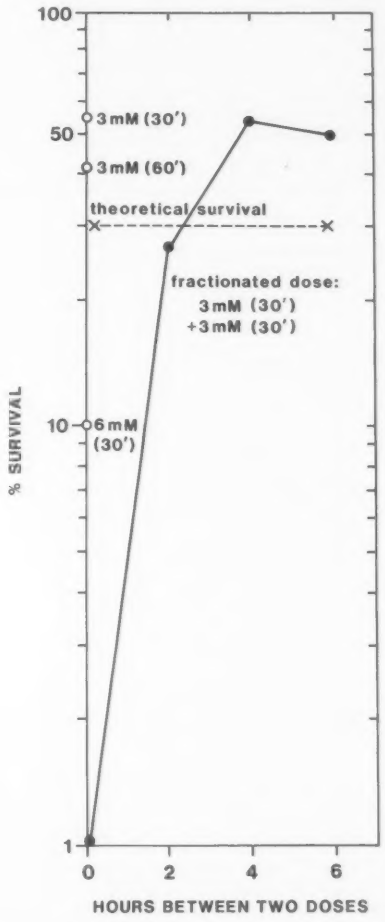

FIGURE 6.—Survival of plateau-phase hepatoma 3924A cells after total (6 mM for 30 mins or 3 mM for 60 mins), $^1/_2$ of total (3 mM for 30 mins), and fractionated-dose treatment with VF 122 (●). Cells that received fractionated doses were exposed to a single dose of 3 mM VF 122 for 30 mins at 0 time. During the interval between receiving 2 doses, cells were incubated in depleted plateau-phase medium. Other experimental conditions and evaluations of results were as in fig 5. Each point is the average of triplicate samples of 3 experiments.

TABLE 1.—Recovery of plateau-phase hepatoma 3924A cells from PLD

Treatment at 0 time		% survival after incubation* for—			
VF 122 (mM)	Incubation (mins)	0 hrs	2 hrs	4 hrs	6 hrs
3.0	30	55	58	66†	76†
3.0	60	42	59†	73†	80†
6.0	30	10	25†	34†	52†
6.0	60	2	77†	53†	75†

*Cells were incubated in depleted 96-hr plateau-phase medium for increasing time periods. Mean values of triplicate determinations are given.

†Significantly higher survival compared to cells plated immediately (0 time) ($P < 0.05$).

same drug treatment; after removal of the drug, the cells were incubated in depleted plateau-phase medium for different periods before seeding for colony formation. After 6 hrs of incubation, the survival values were significantly higher (80% for 1 mM VF 122 and 63% for 2 mM VF 122) than those of cells plated immediately after the treatment. These results clearly indicated the ability of exponentially growing cells to recover from PLD. Plateau-phase cells were treated and assayed in the same manner; they exhibited similar ability to repair PLD. These data are presented in table 1.

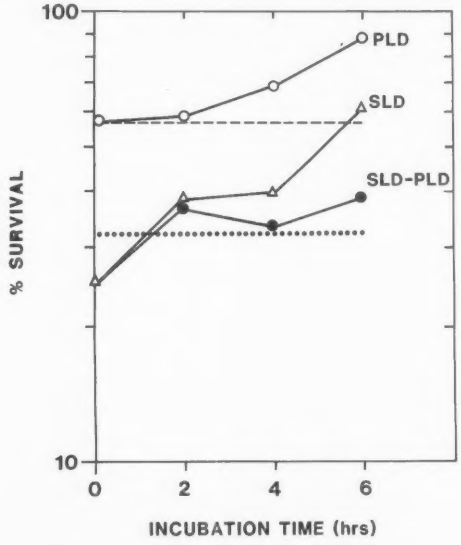

FIGURE 7.—Real recovery of exponentially growing hepatoma 3924A cells from SLD. Cells were exposed to fractionated doses of VF 122 (1 mM for 30 mins) to obtain SLD curve described in fig 5. To measure PLD, cells were treated with 1 mM VF 122 for 30 mins at 0 time. 1 group of cells was plated immediately for colony formation. Other groups of cells were washed with PBS and reincubated in fresh medium. At times indicated, cells were plated for colony formation. SLD-PLD curve was obtained by reducing SLD survival points (60% after 6-hr incubation) by the fraction due to recovery from PLD. This fraction was calculated as the difference between PLD survival points obtained after incubation in fresh medium (87% for 6 hrs incubation) and value obtained without incubation (57% survival, indicated by ----). Colonies were counted and results were evaluated as in fig 1. Theoretical survival (·····).

Parallel recovery experiments were also designed to determine the contribution made by PLD recovery to recovery from SLD. For that purpose, exponentially growing cells were treated as for PLD experiments (1 mM VF 122 for 30 mins), but the cells were incubated in fresh medium for 2, 4, or 6 hrs before seeding for colony formation (fig 7). To determine the contribution made by PLD recovery, an SLD-PLD curve was constructed by reducing the SLD survival points by the survival fraction due to recovery from PLD. The fraction that recovered from PLD was calculated by subtracting the value obtained at zero time (no incubation in fresh medium) from the survival values on the PLD curve obtained after incubation for 1–6 hrs (fig 7). The real recovery from fractionated doses of VF 122 is given in figure 7 for exponentially growing cells and in figure 8 for plateau-phase cells.

All these data suggested that part of the observed recovery from SLD was due to recovery from PLD. PLD recovery was high enough to mask the true response to SLD. These data support the observation of Barranco et al (16) that one type of damage (PLD) could be so great as to mask the true response of cells to another type of damage (SLD). Different sensitivities to VF 122 observed between proliferating and resting hepatoma 3924A cells could not be explained with survival responses to SLD. Namely, exponentially growing cells treated with VF 122 did not exhibit a shoulder-like dose-survival curve but they recovered from SLD as well as did plateau-phase cells.

The results presented here might also contribute to the study of the regrowth of tumor cells in in vitro systems, ie, a tissue culture system that is based on a transition from the nonproliferating to the proliferating phase is useful in the study of the neoplastic growth program (29,30).

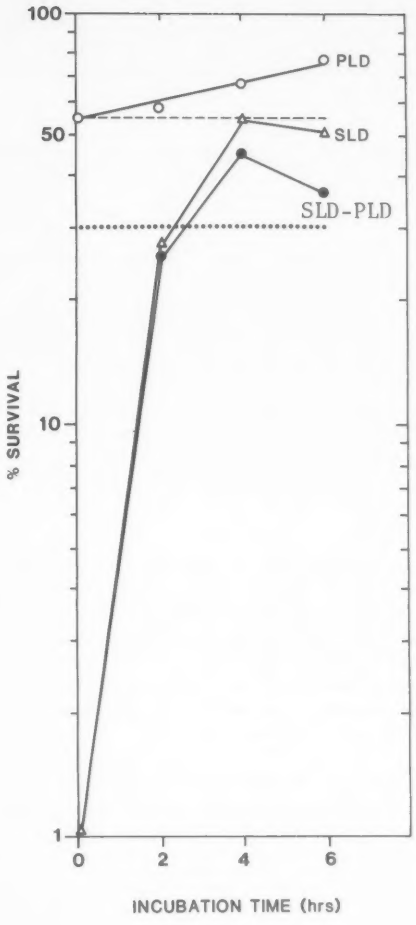

FIGURE 8.—Real recovery of plateau-phase hepatoma 3924A cells from SLD. Cells were exposed to fractionated doses of VF 122 (3 mM for 30 mins) to obtain SLD curve described in fig 6. To measure PLD, cells were treated with 3 mM VF 122 for 30 mins at 0 time. 1 group of cells was plated immediately for colony formation. Other groups of cells were washed with PBS and reincubated in depleted plateau-phase medium. At times indicated, cells were plated for colony formation. Colonies were counted and results were evaluated as in fig 1. Theoretical survival (·····); no incubation in depleted plateau-phase medium(----).

REFERENCES

1. PRATT WB, and RUDDON RW. The anticancer drugs. New York and Oxford, Oxford University Press, 1979, pp 240–245.
2. KRAKOFF IH, BROWN NC, and REICHARD P. Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. Cancer Res 28:1559–1565, 1968.
3. THURMAN WG. Symposium on hydroxyurea. A discussion of pharmacology, mechanism of action, animal screening studies, and chemical studies. Cancer Chemother Rep 40:1–75, 1964.
4. ELFORD H. Effect of hydroxyurea on ribonucleotide reductase. Biochem Biophys Res Commun 33:129–135, 1968.
5. WEBER G. Enzymology of cancer cells. N Engl J Med 296:486–493 and 541–551, 1977.
6. TAKEDA E, and WEBER G. Role of ribonucleotide reductase in expression of the neoplastic program. Life Sci 28:1007–1014, 1981.
7. ELFORD H. Functional regulation of mammalian ribonucleotide reductase. Adv Enzyme Regul 10:19–38, 1972.
8. TURNER MK, ABRAMS R, and LIEBERMAN I. Levels of ribonucleotide reductase activity during the division cycle of the L cell. J Biol Chem 243:3725–3728, 1968.
9. ELFORD HL, WAMPLER GL, and VAN'T RIET B. New ribonucleotide reductase inhibitors with antineoplastic activity. Cancer Res 39:844–851, 1979.
10. VAN'T RIET B, WAMPLER GL, and ELFORD HL. Synthesis of hydroxy- and amino-substituted benzohydroxamic acids: inhibition of ribonucleotide reductase and antitumor activity. J Med Chem 22:589–592, 1979.
11. VAN'T RIET B, KIER LB, and ELFORD HL. Structure activity relationships of benzohydroxamic acid inhibitors of ribonucleotide reductase. J Pharm Sci 69:856–857, 1980.
12. OLAH E, LUI MS, TZENG DY, ET AL. Phase and cell cycle specificity of pyrazofurin action. Cancer Res 40:2869–2875, 1980.
13. TERASIMA T, and TOLMACH LJ. Changes in X-ray sensitivity of HeLa cells during the division cycle. Nature 190:1210–1211, 1961.
14. ———.Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. Exp Cell Res 30:344–362, 1963.
15. BAN J, OLAH E, and WEBER G. Action of lycurim and pyrazofurin on hepatoma 3924A cells in culture. Cancer Treat Rep 66:343–349, 1982.
16. BARRANCO SC, NOVAK JK, and HUMPHREY RM. Studies on recovery from chemically induced damage in mammalian cells. Cancer Res 35:1194–1204, 1975.
17. DREWINKO B. Cellular pharmacology. *In* Cancer and Chemotherapy, vol 1, (Crooke ST, and Prestoyko AW, eds). New York, Academic Press, Inc, 1980, pp 95–120.
18. BRUCE WR, MEEKER BE, and VALERIOTE FA. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered in vivo. JNCI 37:233–245, 1966.
19. HILL BT. Cancer chemotherapy. The relevance of certain concepts of cell cycle kinetics. Biochim Biophys Acta 516:389–417, 1978.
20. BARRANCO SC, HAENELT BR, and GEE EL. Differential sensitivities of five rat hepatoma cell lines to anticancer drugs. Cancer Res 38:656–660, 1978.
21. BARRANCO SC, and NOVAK JK. Survival responses of dividing and nondividing mammalian cells after treatment with hydroxyurea, arabinosylcytosine, or adriamycin. Cancer Res 34:1616–1618, 1974.
22. BHUYAN BK, FRASER TJ, and DAY KJ. Cell proliferation kinetics and drug sensitivity of exponential and stationary populations of cultured L1210 cells. Cancer Res 37:1057–1063, 1977.
23. MAURO F, FALPO B, BRIGANTI G, ET AL. Effects of antineoplastic drugs on plateau-phase cultures of mammalian cells. Bleomycin and hydroxyurea. JNCI 52:715–721, 1974.
24. BACCHETTI S, and WHITMORE GF. The action of hydroxyurea on mouse L-cells. Cell Tissue Kinet 2:193–211, 1969.
25. OLAH E, PALYI I, and SUGAR J. Effects of cytostatics on proliferating and stationary cultures of mammalian cells. Eur J Cancer 14:895–900, 1978.
26. SINCLAIR WK. Hydroxyurea: effects on Chinese hamster cells grown in culture. Cancer Res 27:297–308, 1967.

27. LITTLE JB. Repair of sublethal and potentially lethal radiation damage in plateau phase cultures of human cells. Nature 224:804–806, 1969.
28. BELLI JA, and SHELTON M. Potentially lethal radiation damage: repair by mammalian cells in culture. Science 165:490–492, 1969.
29. WEBER G, OLAH E, LUI MS, ET AL. Biochemical commitment to replication in cancer cells. Adv Enzyme Regul 18:3–26, 1980.
30. WEBER G, OLAH E, DENTON JE, ET AL. Dynamics of modulation of biochemical programs in cancer cells. Adv Enzyme Regul 19:87–102, 1981.

Prednimustine in Combination With Methotrexate and 5-FU (PMF): A Pilot Study[1]

Else Boesen and H. T. Mouridsen[2,*]

A group of 47 patients with advanced breast cancer were treated with a combination of prednimustine, methotrexate, 5-FU, and tamoxifen. In this combination, with methotrexate and 5-FU administered at standard doses, hematologic toxicity of prednimustine at a dose of 100 mg/m² orally on Days 1–5 repeated every 4 weeks was acceptable. Nausea and vomiting occurred in 40 of the 47 patients but was only grade 1–2 in 35 of these patients. Only one patient had alopecia to the extent of requiring a wig. Response to treatment could be evaluated in 28 patients; 21 achieved a response (complete or partial) lasting a median of 13 months. [Cancer Treat Rep 66:2081–2083, 1982]

Prednimustine is an ester of chlorambucil and prednisolone. In a previous phase II study an intermittent schedule of prednimustine proved to be active in advanced breast cancer (1), and in a recent phase III study both intermittent and continuous schedules of prednimustine were demonstrated to be superior to continuous treatment with the combination of chlorambucil plus prednisolone (2). CMF (cyclophosphamide, methotrexate, and 5-FU) has been one of the most widely used combination therapies in advanced breast cancer, with a response rate of about 50% (3,4), and recent studies have indicated that the response rate is further increased when tamoxifen is added to the combination (5,6).

The obvious objective of studies in advanced breast cancer is to develop regimens with higher efficacy and/or lower toxicity. Based on the available data in breast cancer it seems that prednimustine may be at least as effective as cyclophosphamide and that it probably causes less subjective toxicity and a lower grade of alopecia (2). Therefore, a randomized trial to compare CMF with a combination of prednimustine, methotrexate, and 5-FU (PMF) seems reasonable. However, preliminary experience with PMF is first needed.

The objectives of the present study were primarily to analyze the tolerability of prednimustine administered at different dose levels in combination with methotrexate, 5-FU, and tamoxifen, and secondarily to analyze the efficacy of this combination.

MATERIALS AND METHODS

Patients.—Postmenopausal patients ≤ 68 years of age with advanced progressive breast cancer, with or without measurable or evaluable lesions, were entered in the trial, provided they had not received prior cytotoxic therapy, had normal bone marrow function as defined by a wbc count > 4000/μl and a platelet count ≥ 100,000/μl, had no other previous or concomitant malignant disease, and had no glucosuria or symptoms of active gastric or duodenal ulcer. Oral informed consent was obtained.

Treatments.—Two consecutive series (1 and 2) of patients who fulfilled the criteria of eligibility were treated with PMF. Patients in series 1 received the following doses per cycle: prednimustine, 80 mg/m² orally on Days 1–5; methotrexate, 40 mg/m² iv on Days 1 and 8; and 5-FU, 600 mg/m² iv on Days 1 and 8. Patients in series 2 received 100 mg/m² of prednimustine orally on Days 1–5 and doses of methotrexate and 5-FU as in series 1. In both series the cycles were repeated every 4 weeks. In addition, all but four patients received 20 mg of tamoxifen twice daily.

Assessment of hematologic toxicity and adjustment of dose.—During the first three cycles wbc and platelet counts were measured weekly. Thereafter, they were measured only on Days 1 and 8 of each cycle. Doses were adjusted for hematologic toxicity on Days 1 and 8 of each cycle according to the following values for wbc

[1]Received Mar 8, 1982; revised Aug 26, 1982; accepted Sept 22, 1982.

[2]Department of Oncology A, Finsen Institute, Copenhagen, Denmark.

*Reprint requests to: H. T. Mouridsen, MD, Department of Oncology A, Finsen Institute, 49 Strandboulevarden, DK-2100 Copenhagen Ø, Denmark.

TABLE 1.—Wbc and platelet count nadirs in patients given PMF in series 1 and 2*

Toxicity grade†	No. of patients in series 1 or 2											
	Cycle 1				Cycle 2				Cycle 3			
	Wbc count		Platelet count		Wbc count		Platelet count		Wbc count		Platelet count	
	1	2	1	2	1	2	1	2	1	2	1	2
1	18	12	23	18	11	7	23	18	9	6	18	18
2	6	7	2	1	10	9	1	0	9	10	2	1
3	1	0	0	1	4	3	1	1	3	3	1	0
4	1	2	1	1	1	1	1	1	0	0	0	0

*Series 1 = 26 patients; prednimustine dose, 80 mg/m^2. Series 2 = 21 patients; prednimustine dose, 100 mg/m^2.

†Toxicity grades according to wbc and platelet count, respectively ($\times 10^3/\mu l$): 1, ≥ 3.0 and ≥ 100; 2, 2.0–2.9 and/or 75–99; 3, 1.0–1.9 and/or 50–74; and 4, < 1.0 and/or < 50.

($\times 10^3/\mu l$) and platelet ($\times 10^3/\mu l$) counts: ≥ 3 and ≥ 100, 100% of dose; 2.0–2.9 and/or 75–99, 50% of dose; 1.0–1.9 and/or 50–74, 25% of dose; and < 1.0 and/or < 50, no drug.

Assessment of nonhematologic toxic effects.—The nonhematologic toxic effects were graded 0–4, 0 indicating absent, 1 indicating mild, 2 indicating moderate, 3 indicating severe, and 4 indicating life-threatening toxicity according to World Health Organization criteria (7).

Assessment of response to treatment.—Response to treatment was analyzed, according to the International Union Against Cancer criteria (8), in patients with measurable and/or evaluable lesions who had received a minimum of two cycles.

RESULTS

From June 1979 to April 1980 a total of 47 patients entered the trial, 26 in series 1 and 21 in series 2.

Hematologic toxicity and dose adjustment.—The wbc and platelet count nadirs during the first three cycles in series 1 and 2 are given in table 1. It appears that the wbc counts were depressed significantly more often than platelet counts, and it further appears that during the first three cycles 70% of the patients had a wbc count nadir corresponding to toxicity grade 2 or more. In both series, the doses of prednimustine, methotrexate, and 5-FU had to be reduced because of toxic effects, more so in series 2 (table 2). A total of 38 patients were treated for an additional 1–19 months (median, 10.5). No signs of cumulative hematologic toxicity were observed in these patients, and median drug doses in cycles 5, 10, and 15 were 74%, 77%, and 72%, respectively. Three patients developed severe leukopenia and sepsis requiring admission to hospital and treatment with antibiotics.

Nonhematologic toxic effects.—Nausea and vomiting occurred in 40 of the 47 patients; five patients had grade 3–4 and 35 had grade 1–2. Only one of the patients developed alopecia to the extent of requiring a wig. One patient developed extensive condylomata during treatment and another developed upper abdominal pain which subsided on further treatment when cyclophosphamide was substituted for prednimustine. Gastroscopy in this patient was normal.

Response to treatment.—Response could not be analyzed in 19 patients. Ten patients did not have measurable or evaluable disease. Another four patients did not receive tamoxifen, two received only one cycle, one was lost to follow-up, and two had a previously treated carcinoma of the cervix but without signs of recurrence of that disease. In these 19 patients we analyzed only the time until progression. Among the other 28 patients with evaluable disease (16 in series 1 and 12 in series 2), complete remission was observed in 12 cases and partial remission in nine cases, giving a total remission rate of 75% with a median duration of response of 13 months. No change was observed in five patients, and progressive disease was seen in two patients. Among the nine responding patients in series 1, the dominant sites of lesion were: soft tissue (six patients), viscera

TABLE 2.—Average relative drug doses

Series	% of dose					
	Cycle 1		Cycle 2		Cycle 3	
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
1	98	74	83	75	84	79
2	94	83	84	69	76	69

(two), and bone (one). The dominant sites in the 12 responding patients in series 2 were: soft tissue (four), viscera (five), and bone (three). The median time until progression was 11 months in the 28 patients with evaluable disease, compared to 8 months in the 19 patients with unevaluable disease.

DISCUSSION

The major objective of the present study was to analyze the toxicity of prednimustine when combined with methotrexate and 5-FU. In the PMF combination, with methotrexate and 5-FU administered at standard doses, hematologic toxicity of prednimustine at a dose of 100 mg/m^2 on Days 1–5 was acceptable. Gastrointestinal symptoms were less pronounced with PMF than normally reported with CMF (6), and only one of the 47 patients developed alopecia to the extent of requiring a wig, which definitely seems to be less frequent than with the CMF combination.

Response to treatment could be evaluated in 28 patients, 21 (75%) of whom responded with a 13-month median duration of response. These figures are in agreement with results achieved with the CMF plus tamoxifen combination (6).

In conclusion, it seems that prednimustine can be safely used in combination with methotrexate and 5-FU. The frequency of alopecia is definitely lower with PMF than with CMF. Conclusions as to whether this relates also to subjective side effects will require a randomized study, as will a final conclusion concerning the efficacy of PMF compared to that of CMF.

REFERENCES

1. Mouridsen HT, Kristensen D, Nielsen JH, et al. Phase II trial of prednimustine L-1031, (NSC 134087) in advanced breast cancer. Cancer 46:254–255, 1980.
2. Løber J, Mouridsen HT, Christiansen IE, et al. A phase III trial comparing prednimustine (Leo 1031) to chlorambucil plus prednisolone in advanced breast cancer. Cancer. In press.
3. Carter SK. Integration of chemotherapy into combined treatment of solid tumors. VII. Adeno-carcinoma of the breast. Cancer Treat Rev 3:141–174, 1976.
4. Rozencweig M, Heuson JC, Von Hoff DD, et al. Breast cancer. *In* Randomized Trials in Cancer. A Critical Review by Sites (Staquet MJ, ed). New York, Raven Press, 1978, p 231.
5. Mouridsen HT, Palshof T, Engelsman E, et al. CMF versus CMF plus tamoxifen in advanced breast cancer in postmenopausal women. An EORTC trial. Eur J Cancer (suppl) 119–123, 1980.
6. Cocconi G, Leasi BD, Boni C, et al. Chemotherapy versus combination of chemotherapy and endocrine therapy in advanced breast cancer. A prospective randomized study. UICC Conference on Clinical Oncology. Breast Cancer. Abstr 03–0141, 1981.
7. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, WHO Offset Publication No. 48, 1979.
8. Hayward JL, Carbone PP, Heuson J-C, et al. Assessment of response to therapy in advanced breast cancer. Cancer 39:1289–1294, 1977.

Cellular and Plasma Kinetics of Daunorubicin Given by Two Methods of Administration in a Patient With Acute Leukemia[1,2]

Michael W. DeGregorio,* Carlos J. Carrera, John C. Klock, Charles H. Pegelow, and Jordan R. Wilbur[3,4]

Leukemic cell and plasma concentrations of daunorubicin were examined serially in a patient with acute lymphoblastic leukemia following administration of the drug by two different regimens: regimen A—an iv bolus dose of 50 mg/m², and regimen B—a loading dose of 15 mg/m² followed by 35 mg/m² infused over 4 hours. Peak plasma levels were four times higher after regimen A than B, whereas leukemic cell concentrations were not significantly different. No difference in acute toxicity or therapeutic efficacy was seen after either method of administration. However, we have demonstrated that peak plasma levels of daunorubicin can be reduced by continuous infusion without significantly altering target tissue concentrations. [Cancer Treat Rep 66:2085–2088, 1982]

The anthracycline antibiotics doxorubicin (DOX) and daunorubicin (DNR) are among the most important chemotherapeutic agents currently used in the treatment of patients with acute leukemia (1). These drugs are usually administered by a bolus dose over 3–5 minutes. In addition to the toxic effects common to most cancer drugs (bone marrow suppression, alopecia, stomatitis, nausea, and vomiting), the effective use of anthracyclines is limited by the unique toxicity of cardiomyopathy related to the cumulative dose of these drugs (2,3). Patients who could benefit from the continued use of anthracycline therapy may not be able to receive this treatment because of either the risk or actual development of life-threatening cardiac disease. Two studies have shown that altering the dosage schedules of DOX has reduced the incidence of cardiotoxicity and has allowed higher cumulative doses (4,5). The lowering of peak plasma levels may be one factor responsible for the reduction of DOX-induced cardiotoxicity (5).

We examined the cellular and plasma kinetics of DNR in a patient with acute leukemia after a bolus dose and again after a small iv loading dose followed by a 4-hour continuous infusion. The purpose of this preliminary study was to determine whether altering the method of administration would affect the cellular and plasma kinetics of DNR.

Patient History

The patient is a 26-year-old white male with acute lymphoblastic leukemia in his third relapse who had normal liver and renal functions (creatinine < 1.3 mg/100 ml) during the study periods. Past induction regimens included vincristine, prednisone, L-asparaginase, DNR, and cytarabine. At this time, remission induction was attempted using a combination of cyclophosphamide, vincristine, DNR, and prednisone. To avoid possible drug-drug interaction, cyclophosphamide was not administered within 24 hours of the DNR doses. The total cumulative dose of DNR was 225 mg/m² before bolus therapy and 275 mg/m² before the small bolus followed by short continuous infusion. Just prior to the bolus dose of 50 mg/m² of DNR, the peripheral blast

[1]Received Apr 15, 1982; revised Sept 14, 1982; accepted Sept 28, 1982.

[2]Supported by the Louis R. Lurie Foundation, the Kent Bourne Memorial Fund, University of California Cancer Research Coordinating Committee account, and the American Society of Hospital Pharmacist Research and Education Foundation Grant.

[3]Children's Cancer Research Institute (*M. W. DeGregorio, C. H. Pegelow,* and *J. R. Wilbur*), Institute of Cancer Research (*M. W. DeGregorio* and *J. C. Klock*), Pacific Medical Center, and the Cancer Research Institute, University of California (*M. W. DeGregorio* and *C. J. Carrera*), San Francisco.

[4]We thank Maria S. Drake for technical assistance and James Harris for typing this manuscript.

**Reprint requests to:* Dr. Michael DeGregorio, Children's Cancer Research Institute, 2351 Clay St, S-512, Pacific Medical Center, San Francisco, CA 94115.

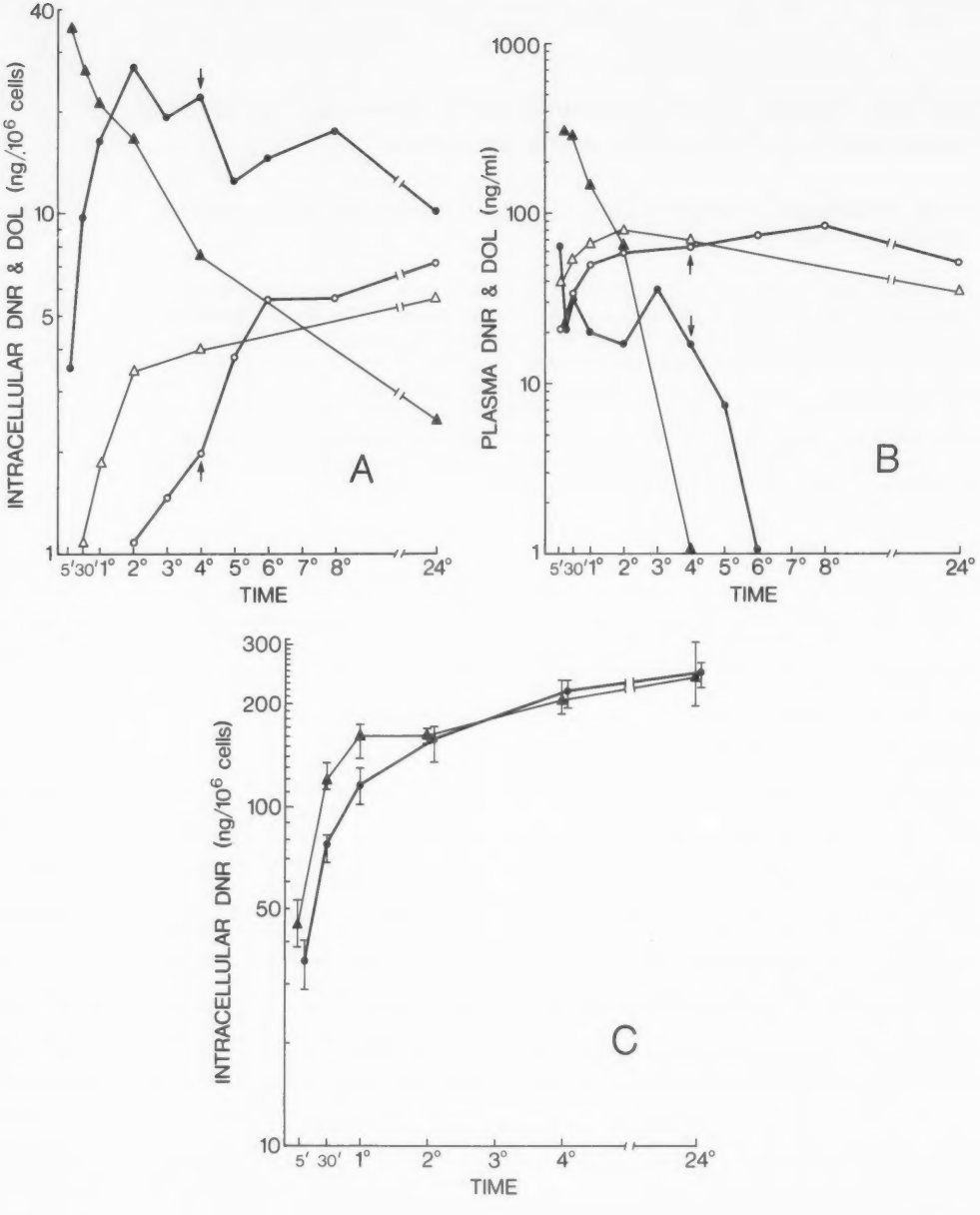

FIGURE 1.—Leukemic cell (A) and plasma (B) concentrations of DNR (▲) and DOL (Δ) after bolus administration, and DNR (●) and DOL (○) during and after the 4-hr infusion. Arrows indicate end of infusion. C. In vitro uptake of DNR by leukemic cells before bolus injection (▲) and before continuous infusion (●).

count was 134,000/mm^3. The peripheral blast count nadir 7 days after therapy was 37,000/mm^3 and thereafter quickly rose to 74,000/mm^3. A second course of cyclophosphamide, vincristine, DNR, and prednisone was instituted in the same manner with the exception that DNR was administered by a loading dose of 15 mg/m^2 followed by a 35-mg/m^2 infusion over 4 hours. This resulted in a decrease of the peripheral blast count to a nadir of 18,000/mm^3 8 days after therapy.

MATERIALS AND METHODS

Blood samples were anticoagulated with EDTA at several time points and rapidly cooled to 4°C. Each sample was centrifuged at 200 × *g* for 10–15 minutes and plasma was removed. The resulting plasma was centrifuged a second time at 1040 × *g* for 5–10 minutes to remove remaining leukocytes. The buffy coat was depleted of erythrocytes by cold hypotonic lysis. Remaining leukocytes (> 98% lymphoblasts) were then resuspended in 2 ml of isotonic saline and the cell concentration was determined by a Coulter counter. Samples (1-ml) of plasma and cell suspensions were then processed for higher performance liquid chromatography (HPLC) analysis.

Quantitation of leukemic cell and plasma concentration of DNR was performed utilizing HPLC methods previously reported (6). To summarize: an internal standard DOX was added to plasma and cell samples which were then extracted using a chloroform/methanol mixture. Extracted samples were stored at –20°C in the dark until the day of analysis. Samples were reconstituted with 100 μl of chloroform and 25-μl aliquots were injected for analysis.

In vitro uptake and metabolism of DNR by the patient's leukemic cells were determined as previously described (7). To summarize: leukemic cells before each treatment were isolated from peripheral blood using Ficoll-Hypaque density gradient centrifugation. The viability was assessed before and after each experiment by erythrosin-B dye exclusion. Leukemic cells suspended in RPMI-1640 medium enriched with 15% fetal calf serum were exposed to ^{14}C-radiolabeled DNR at a test concentration of 1 μg/ml. Cells were incubated at 37°C and at several time points triplicate samples were assayed for intracellular DNR concentration by liquid scintillation spectrometry.

RESULTS

The cellular and plasma concentrations of DNR and daunorubicinol (DOL) resulting from the two methods of drug administration are shown in figure 1, A and B. Plasma concentrations of DNR declined rapidly following both the bolus dose and the end of the infusion. The peak plasma concentrations of DNR during infusion were approximately 20%–25% of the peak achieved after the bolus dose. Intracellular DNR levels after the bolus dose also declined rapidly, whereas the leukemic cell levels of DNR increased during the period of infusion and actually exceeded intracellular levels achieved after the bolus dose. Plasma and leukemic cell concentrations of DOL were not significantly different after either method of administration.

The in vitro uptake of DNR by the leukemic cells before each treatment is shown in figure 1C. No significant difference in the pattern of in vitro uptake was seen. The in vitro metabolism of DNR as determined by HPLC techniques was not significant (< 5%) in either study after 24-hour incubation with 1 μg/ml.

DISCUSSION

Our results show that compared to bolus therapy, the administration of DNR by short continuous infusion reduces peak plasma levels but achieves similar drug concentrations in the leukemic cells.

Experimental data in animal models suggest that the administration of anthracyclines by repeated small doses results in lower levels of drug in cardiac tissues compared to the same total dose given as a single bolus (8). Legha et al (5) have recently reported reduced cardiotoxicity in cancer patients who had received DOX as a prolonged continuous infusion. These authors speculated that this lower toxicity was due to a reduction in peak plasma levels of DOX during infusion as opposed to those associated with standard bolus therapy. Similar studies using DNR in patients with leukemia have not been reported.

The in vitro uptake of DNR and other anthracyclines by leukemic cells has been reported by several investigators (9,10). Maximal intracellular DNR concentrations are achieved between 2 and 4 hours after continuous in vitro drug exposure. Similarly, the in vitro uptake patterns of the cells isolated from our patient before each regimen demonstrated maximal DNR uptake after 4 hours of exposure. Based on these in vitro findings, we elected to give a small bolus dose followed by a 4-hour continuous infusion to maximize DNR uptake by the leukemic cells in vivo. Clearly, the cellular uptake pattern during DNR infusion mimics that seen in vitro.

In conclusion, we have demonstrated that a reduction in peak plasma levels of DNR can be achieved without altering intracellular drug concentrations by using a different method of DNR administration.

REFERENCES

1. Young RC, Ozols RF, and Myers CE. The anthracycline antineoplastic drugs. N Engl J Med 305:139–153, 1981.

2. Von Hoff DD, Layard MW, Basa P, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91:710–717, 1979.
3. Minow RA, Benjamin RS, Lee ET, et al. Adriamycin cardiomyopathy: risk factors: Cancer 39:1397–1402, 1977.
4. Weiss AJ, and Manthel RW. Experience with the use of adriamycin in combination with other anticancer agents using a weekly schedule, with particular reference to lack of cardiac toxicity. Cancer 40:2046–2052, 1977.
5. Legha SS, Benjamin RS, Mackay B, et al. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 96:133–139, 1982.
6. Andrews P, Brenner D, Chou F, et al. Facile and definitive determination of human adriamycin and daunomycin metabolites by high-pressure liquid chromatography. Drug Metab Dispos 8:152–156, 1980.
7. DeGregorio MW, Carrera CJ, Klock JC, et al. Uptake and metabolism of daunorubicin by human leukemia cells. Cancer Chemother Pharmacol. In press.
8. Pacciarini MA, Barbieri B, Colombo T, et al. Distribution and antitumor activity of adriamycin given in a high-dose and repeated low-dose schedule to mice. Cancer Treat Rep 62:791–800, 1978.
9. Bachur NR, Steele M, Meriwether WD, et al. Cellular pharmacodynamics of several anthracycline antibiotics. J Med Chem 19:651–654, 1976.
10. Yesair DW, Thayer PS, McNitt S, et al. Comparative uptake, metabolism and retention of anthracyclines by tumors growing in vitro and in vivo. Eur J Cancer 16:901–907, 1980.

Phase II Study of an Aziridinylbenzoquinone (AZQ) in Disseminated Malignant Melanoma[1]

E. T. Creagan,* A. J. Schutt, D. L. Ahmann, and S. J. Green[2]

Thirty-four patients with advanced malignant melanoma received AZQ (an aziridinylbenzoquinone) at a dose of 22.5 or 27.5 mg/m²/month iv. Objective regressions among 23 previously untreated and 11 previously treated patients were two and none, respectively. Hematologic toxicity was dose-limiting. [Cancer Treat Rep 66:2089–2090, 1982]

Clinically meaningful chemotherapy for patients with advanced malignant melanoma remains elusive. Dacarbazine and the nitrosoureas achieve response rates of only 15%–20% (1,2). Combination regimens have not consistently enhanced either the probability or the duration of objective regressions (1). In this setting, AZQ, an aziridinylbenzoquinone, seemed to be appropriate for clinical trials in advanced melanoma. In animal tumor models, striking activity was noted in an array of solid neoplasms, including Walker carcinosarcoma 256, adenocarcinoma 755, and Sarcoma 180, and in L1210 leukemia (3). In the B16 melanoma murine tumor system, AZQ produced a 140% increase in survival compared with controls (4). Of particular interest are data from primates clearly showing that AZQ has a prolonged concentration in cerebrospinal fluid comparable to plasma levels (5). This is an attractive characteristic of AZQ in light of the alarming frequency of CNS metastases from melanoma.

From a phase I study of our institution, the dose-limiting toxicity was myelosuppression, with clinically acceptable gastrointestinal toxicity. A dose of 27.5 mg/m²/month produced erratic, occasionally cumulative myelosuppression, especially in patients with prior chemotherapy and/or radiation therapy. For the latter group, a dose of 22.5 mg/m²/month was deemed appropriate. We report here a phase II study of AZQ in patients with advanced malignant melanoma.

MATERIALS AND METHODS

Thirty-four patients with biopsy-confirmed disseminated malignant melanoma participated in the clinical trial. There were 23 males and 11 females, with a median age of 49 years. Eleven patients had received prior chemotherapy and 23 had had no prior systemic cytotoxic treatment. Eastern Cooperative Oncology Group performance scores (PS) were 0 or 1 (29 patients) or 2 or 3 (five patients). Nineteen patients had visceral-dominant disease, including five with known or suspected liver involvement. Fifteen patients had soft tissue or nodal involvement.

Drug Administration

AZQ was administered iv over 10–15 minutes at a dose of 27.5 mg/m² every 4 weeks for the 14 good-risk patients (< 70 years old, PS ≤ 2, no prior chemotherapy, and no known or suspected liver metastases). An AZQ dose of 22.5 mg/m²/month iv was used for poor-risk patients (≥ 70 years old, debilitated, prior chemotherapy, or liver involvement).

Evaluation

Prior to initial treatment and subsequent courses, a thorough general medical assessment, routine tests of hematologic and chemical parameters, chest X-ray, and photographs of appropriate lesions were done for each patient.

An objective response was defined as a decrease ≥ 50% in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion. There must have been no new areas of malignant disease or symptomatic deterioration. Progressive disease was defined as a > 25% increase in any indicator lesion or the appearance of new lesions. We analyzed eight patients with evaluable but not clearly measurable dis-

[1]Received June 1, 1982; revised Aug 16, 1982; accepted Sept 21, 1982.
[2]Division of Medical Oncology (*E. T. Creagan, A. J. Schutt,* and *D. L. Ahmann*), and Cancer Center Statistics (*S. J. Green*), Mayo Clinic, Rochester, MN.
Reprint requests to: E. T. Creagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

ease using previously reported criteria showing that "assessable but nonmeasurable disease" can provide reliable and consistent response and survival data (6). We calculated the time to disease progression from the first day of treatment to date of progression. Seven patients did not return for evaluation after the first treatment; local physicians confirmed progression of disease in six of these patients, and one patient was assumed to have had progressive disease on the date of scheduled return.

RESULTS

Therapeutic (table 1).—Among 23 patients with no prior chemotherapy, one experienced a partial regression of nodal and subcutaneous nodules (time to progression, 3.5 months) and one had a complete response of cervical and axillary lymphadenopathy (7 months) (table 1). These two patients were among our 14 "best-risk patients" (no prior chemotherapy, PS of 0 or 1, < 70 years old, and no liver involvement). For all 34 study participants, the median times to progression and survival were 1 and 5 months, respectively (Kaplan-Meier method).

Toxicity.—Toxic effects were primarily hematologic. Among 26 patients with interim blood tests, four (15%) had wbc counts $\leq 1.5 \times 10^3$ cells/mm^3. The median wbc count nadir was 4.0×10^3 cells/mm^3 (range, 1.1–6.5). Six patients (23%) with interim platelet counts had thrombocytopenia ($< 75 \times 10^3$ cells/mm^3). The median platelet count was 150×10^3 cells/mm^3 (range, 33–370). There were no significant episodes of sepsis or hemorrhage. Mild-moderate and transient nausea and vomiting affected 10% of 34 patients; two other patients experienced severe nausea and vomiting. Other toxic effects included alopecia (one patient with mild, one complete), stomatitis (two with mild-moderate), and constipation (five with mild-moderate).

TABLE 1.—Results of treatment by prior chemotherapy exposure and site of metastasis*

Treatment	Visceral-dominant	Nonvisceral
Primary	0/14	2/9
Secondary	0/5	0/6

*Values = No. of objective regressions/No. of evaluable patients.

DISCUSSION

In this phase II study of AZQ in advanced malignant melanoma, we achieved a response rate of 9% with acceptable toxicity among 23 patients without prior chemotherapy. A 95% confidence level for the probability of response to AZQ in patients with no prior treatment is 0.01, 0.28. The observed responses were confined to soft tissue or nodal sites, were of marginal benefit, and had no impact on visceral metastases. Therefore, it seems unlikely that AZQ, in the dose and schedule that we used, will meaningfully alter the presently inexorable course of disseminated melanoma. The absence of antitumor activity among 11 previously treated patients is consistent with findings by other investigators who observed only a 2% response rate from secondary chemotherapy in advanced melanoma (1). No responses among 11 patients is inconsistent, at the 0.05 level, with a true response probability of > 25% from AZQ in this patient population.

REFERENCES

1. COSTANZA ME, NATHANSON L, SCHOENFELD D, ET AL. Results with methyl-CCNU and DTIC in metastatic melanoma. Cancer 40:1010–1015, 1977.
2. AHMANN DL. Nitrosoureas in the management of disseminated malignant melanoma. Cancer Treat Rep 60:747–751, 1976.
3. DRISCOLL JS, HAZARD GF, JR, WOOD HB, JR, ET AL. Structure-antitumor activity relationships among quinone derivatives. Cancer Chemother Rep 4:1–27, 1974.
4. DRISCOLL JS, DUDECK L, CONGLETON G, ET AL. Potential CNS antitumor agents VI: aziridinylbenzoquinones III. J Pharmacol Sci 68:185–188, 1979.
5. GORMLEY PE, WOOD JH, and POPLACK DG. Ability of a new antitumor agent, AZQ, to penetrate into cerebrospinal fluid. Pharmacology 22:196–198, 1981.
6. EAGAN RT, FLEMING TR, and SCHOONOVER V. Evaluation of response criteria in advanced lung cancer. Cancer 44:1125–1128, 1979.

Phase II Trial of PALA in Hypernephroma and Urinary Bladder Cancer[1,2]

Ronald B. Natale, Alan Yagoda,* David P. Kelsen, Richard J. Gralla, and Robin C. Watson[3]

PALA, a transition-state analog for aspartate transcarbamylase and a potent inhibitor of de novo pyrimidine biosynthesis, demonstrated antitumor activity against several experimental murine solid tumor models, including the FANFT carcinogen-induced bladder carcinoma (1,2). In the FANFT model, PALA was effective alone, and showed marked synergism when combined with cisplatin (2). In phase I studies, PALA given in a weekly schedule was nonmyelosuppressive, produced manageable gastrointestinal (mucositis/diarrhea) and cutaneous (dermatitis) toxic effects, and induced two minor responses in patients with advanced refractory bladder cancer (3). Therefore, a disease-oriented phase II trial of PALA in patients with objectively measurable metastatic urogenital (bladder or renal) cancers was initiated and forms the basis of this report.

MATERIALS AND METHODS

Thirty-three patients (18 with bladder cancer and 15 with hypernephroma) received PALA. Eligibility criteria included histologically confirmed and bidimensionally measurable metastatic disease, Karnofsky performance status (KPS) $\geq$ 50%, wbc count > 4000/mm^3, platelet count > 100,000/mm^3, serum bilirubin < 2.0 mg/100 ml, no chemotherapy in the preceding 3 weeks, and informed consent. A normal electroencephalogram and brain scan by radionuclide or by computerized tomography (CT) were required of the last 14 patients prior to protocol because brain metastases seemed to be a risk factor for PALA-induced CNS toxicity (4). Initially, all patients had a complete history and physical examination, an automated blood cell and platelet count, a 12-channel biochemical screening profile, a serum creatinine (Cr), and a chest roentgenogram. Serum carcinoembryonic antigen (CEA) levels were obtained in 27 patients (17 with bladder cancer and ten with renal cancer), radionuclide liver scans were performed in patients with malignant hepatomegaly and/or abnormal liver function tests, and CT scans were used to evaluate objectively abdominal and pelvic masses. Physical examination and blood cell and platelet counts were performed every week; chest roentgenograms, liver function tests, and serum CEA levels (if elevated) were performed every 3–4 weeks; and liver and abdominal/pelvic CT scans were performed every 4–6 weeks. Patient characteristics are shown in table 1. There were 25 males and eight females, with a median age of 61 years (range, 22–73) and a median KPS of 60% (range, 50%–100%). Except for a patient with bladder cancer, all were quite heavily pretreated, having received a median of three drugs (range, one to five) prior to pro-

TABLE 1.—Patient characteristics

	Bladder cancer	Hypernephroma
Total No. of patients/ No. evaluable	18/17	15/15
KPS		
Median	60%	60%
Range	50%–90%	50%–100%
No. of patients with—		
Complete remission/partial remission	0	0
Minor response	2	0
Prior chemotherapy/hormones	17	7
Prior irradiation	14	3
Prior surgery	10	13
No. of measurable sites		
Lung	9	11
Nodes	7	2
Abdomen/pelvis by CT scan	8	3
Liver	3	2
Skin	1	1
CEA (> 5 mg/100 ml)	9	0

[1]Received Aug 5, 1982; revised Sept 9, 1982; accepted Sept 28, 1982.
[2]Supported in part by contract N01-CM-97274 (Division of Cancer Treatment) and Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and the Aaron Miller-Solid Tumor Service Fund.

[3]Solid Tumor Service (*R. B. Natale, A. Yagoda,* and *D. P. Kelsen*), Developmental Chemotherapy Service, Department of Medicine (*R. J. Gralla*), and Department of Diagnostic Radiology (*R. C. Watson*), Memorial Sloan-Kettering Cancer Center, New York, NY.
**Reprint requests to:* Alan Yagoda, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

tocol. All patients had bidimensional, objectively measurable disease and 13 (eight with bladder cancer and five with renal cancer) had more than one measurable disease parameter for response evaluation.

PALA was administered iv every week as a 45–60-minute infusion. The initial dose was 4.5 g/m^2 for patients with a KPS $\geq$ 70% and normal renal function and 3.75 g/m^2 for patients with a KPS of 50%–60% or minimal renal dysfunction (Cr, 1.9–3.0 mg/dl or a urinary Cr clearance > 50 ml/minute). The dose was escalated by 20% when toxicity was not encountered after three consecutive weekly doses. When severe toxicity occurred, therapy was discontinued until toxicity resolved, and later resumed at a 20%–50% dose reduction. An adequate trial required three doses of PALA with some evidence of toxicity, or two doses with at least moderate toxicity ($\geq$ 3+), as previously defined (3), and 1-month survival. Partial remission was defined as a > 50% decrease in the summed products of the longest perpendicular diameters of all measurable lesions for > 1 month, minor response was defined as a 25%–50% decrease in measurable lesions, stable disease was defined as a < 25% change for $\geq$ 3 months, and disease progression was defined as a > 25% increase or the appearance of new lesions. KPS was used to evaluate subjective changes.

RESULTS

There were no major objective responses in 32 adequately treated and evaluable patients (17 with bladder cancer and 15 with renal cancer). A minor response, consisting of a 30% decrease in metastatic lung nodules, occurred after three doses in two patients with bladder cancer. However, one of these patients developed PALA-related CNS toxic effects (seizures) which required discontinuation of treatment. He died of progressive disease 2 months later. The other patient died of intracerebral hemorrhaging, probably unrelated to PALA, 5 weeks after starting therapy. One patient with bladder cancer is considered to be unevaluable for response because she received only one dose of 3.75 g/m^2, which resulted in extensive severe cutaneous toxicity leading to sepsis and death 24 days after therapy.

The median number of doses was four (range, one to 16). Thirty-two patients had evidence of cutaneous toxicity, which was moderate or severe (3–4+) in 11. Gastrointestinal toxicity occurred in 17 patients and was generally mild (1–2+). Three patients without preceding abnormal CNS signs or symptoms had the sudden onset of seizures after three, four, and five doses of PALA, respectively; brain metastases were discovered on CT scans in all cases. No CNS toxicity occurred in the final 14 patients entered because of the prescreening procedures previously mentioned. Of note, four patients had moderate to severe cutaneous "flare reactions" in previously irradiated areas, typical of the radiation "recall" phenomena occasionally observed with other chemotherapeutic agents, and two additional patients developed bilateral keratoconjunctivitis and corneal ulcerations.

CONCLUSION

PALA given in a weekly schedule lacks significant antitumor activity in patients with advanced bladder cancer (none of 17 patients; 95% confidence limits, 0%–17%) and renal cancer (none of 15; 95% confidence limits, 0%–19%). Although the extent of prior therapy in our bladder cancer population may have predisposed to drug refractoriness, CNS toxicity associated with a weekly PALA schedule discouraged additional patient accrual. Similar qualifications, however, are not required regarding renal cancer because patients were not heavily pretreated. To date, renal cancer has proven to be unresponsive to PALA when given in a 24–120-hour continuous infusion (three patients), a daily × 5 schedule (three patients), or an every-2-week schedule (one patient) (5–7). Including the results of the present series, there have been no significant responses in hypernephroma in 22 adequately treated cases (none of 22 patients; 95% confidence limits, 0%–13%).

REFERENCES

1. Johnson RK, Inouye T, Goldin A, et al. Antitumor activity of *N*-(phosphonacetyl)-L-aspartic acid, a transition-state inhibitor of aspartate transcarbamylase. Cancer Res 36:2720–2725, 1976.
2. Solowey MS, and Murphy WM. Experimental chemotherapy of bladder cancer—systemic and intravesical. Semin Oncol 6:166–183, 1979.
3. Gralla RJ, Casper ES, Natale RB, et al. Phase I trial of PALA. Cancer Treat Rep 64:1301–1305, 1980.
4. Wiley RG, Gralla RJ, Casper ES, et al. Neurotoxicity of the pyrimidine synthesis inhibitor, PALA. Ann Neurol, in press.
5. Hart RD, Ohnuma T, and Holland JF. Initial clinical study of *N*-(phosphonacetyl)-L-aspartic acid (PALA) in patients with advanced cancer. Cancer Treat Rep 64:617–624, 1980.
6. Erlichman C, Strong J, Wiernik P, et al. Phase I trial of PALA (*N*-phosphonacetyl-L-aspartate). Proc Am Assoc Cancer Res and ASCO 20:314, 1979.
7. Valdivieso M, Moore EC, Loo TL, et al. Phase I clinical study of *N*-(phosphonacetyl)-L-aspartate. Proc Am Assoc Cancer Res and ASCO 20:187, 1979.

Combination Chemotherapy in Patients With Advanced Colorectal Carcinoma Previously Treated With 5-FU[1,2]

Harold E. Windschitl,* James E. Krook, Lloyd K. Everson, Stephen A. Cullinan, and Mark Scott[3]

For the North Central Cancer Treatment Group

Semustine, Baker's antifol, and razoxane each have been reported to show activity against colorectal cancer (1–5). For patients failing 5-FU therapy, we elected to study possible combinations of these drugs. We report on a subset of patients entering a clinical trial for advanced colorectal carcinoma conducted by the North Central Cancer Treatment Group.

METHODS

Histologic evidence of neoplastic disease with measurable metastatic lesions, adequate wbc and platelet counts, Eastern Cooperative Oncology Group (ECOG) performance score (PS) < 4, normal nutrition, and previous chemotherapy with 5-FU (on the larger study or not) were required for entry. Prior irradiation or surgery was allowed, but a 4-week interval was required. Patients with elevated bilirubin, cerebrovascular disease, or elevated serum creatinine (limiting conditions) were directly assigned to the semustine plus razoxane program. Patients who had received prior treatment with semustine were assigned to Baker's antifol plus razoxane. Patients having 5-FU, 5-FU plus Baker's antifol, or 5-FU plus razoxane as initial chemotherapy on the larger study, if eligible, were crossed over to razoxane plus Baker's antifol, semustine plus razoxane, or semustine plus Baker's antifol, respectively.

Semustine was given orally at a dose of 150 mg/m^2 on Day 1 regardless of the other drug. Razoxane was given orally at 500 mg/m^2 on Days 2–4 and 29–31 with semustine and at 425 mg/m^2 on Days 1–3 and 29–31 with Baker's antifol. Baker's antifol was given iv over 1 hour at 200 mg/m^2 on Days 1–3 and 29–31 in conjunction with semustine. The dose of Baker's antifol was reduced to 125 mg/m^2 when razoxane was given. Cycles were repeated every 8 weeks. Seventy-seven patients were entered in the study. Two patients were declared ineligible. One patient was not evaluable for response. Table 1 shows patient characteristics. The median age of all patients was 63 years.

RESULTS

Two patients achieved partial response: a crossover patient to Baker's antifol plus razoxane from 5-FU with a PS of 2, as well as a patient with a PS of 0 with limiting conditions who was assigned to semustine plus razoxane. All patients evaluable for response had disease progression and all patients have died. Among randomized patients (as well as a pool of randomized, crossover, and limiting-condition patients) the disease progression times as well as survival times among treatments were not significantly different.

Table 2 shows toxic effects by randomized and nonrandomized patients. Diarrhea was significantly more frequent on one razoxane plus Baker's antifol arm. Both stomatitis and dermatitis were more frequent in the Baker's antifol arms. Thrombocytopenia was more prevalent in the semustine arms.

CONCLUSION

Neither of these two drug combinations offers benefit for the patient with advanced colon or rectal cancer previously treated with 5-FU.

[1]Received Feb 22, 1982; revised Aug 30, 1982; accepted Sept 13, 1982.

[2]Supported by Public Health Service grant CA-25224 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and American Cancer Society grant ND-104A.

[3]This study was conducted by the North Central Cancer Treatment Group. The following institutions participated in the study: St. Cloud Internists, Ltd, MN (*H. E. Windschitl*); The Duluth Clinic, Ltd, MN (*J. E. Krook*); Fargo Clinic, ND (*L. K. Everson*); Illinois Oncology Research Association, Peoria (*S. A. Cullinan*); Mayo Clinic, Rochester, MN (*M. Scott*); Billings Clinic, MT; Central Plains Clinic, Sioux Falls, SD; Grand Forks Clinic, ND; Iowa Oncology Research Association, Des Moines; and Quain and Ramstad Clinic, Bismarck, ND.

**Reprint requests to:* Harold E. Windschitl, MD, St. Cloud Internists, Ltd, 48 N 29th Ave, St. Cloud, MN 56301.

TABLE 1.—Patient characteristics

Pretreatment characteristics	Semustine + razoxane (% of 27 patients)	Semustine + Baker's antifol (% of 22 patients)	Razoxane + Baker's antifol (% of 26 patients)
Type of trial			
Randomized	67	77	38
Nonrandomized	33	23	62
Sex			
Male	52	27	54
Female	48	73	46
ECOG PS*			
0–1	56	59	58
2–3	44	41	42
Site of primary indicator lesions			
Liver	56	50	50
Lung	36	31	34
Other	8	19	16

*0 = fully active; 4 = 100% bedridden.

TABLE 2.—Toxic effects*

Toxic effects	Semustine + razoxane	Semustine + Baker's antifol	Razoxane + Baker's antifol
Nonhematologic	% of 18 R/ % of 9 NR	% of 17 R/ % of 5 NR	% of 10 R/ % of 16 NR
Nausea	61/67	76/60	70/50
Vomiting	50/67	64/40	50/38
Stomatitis	11/0	35/40	20/13
Diarrhea	17/0	18/0	40/44
Dermatitis	17/0	12/0	20/25
Alopecia	0/0	23/0	10/6
Hematologic	% of 16 R/ % of 7 NR	% of 17 R/ % of 5 NR	% of 9 R/ % of 14 R
Leukopenia (cells/mm^3)			
2000–3999	25/29	47/20	45/50
< 2000	44/29	6/40	11/21
Thrombocytopenia (cells/mm^3)			
50,000–99,999	31/29	29/20	0/21
< 50,000	25/0	12/20	0/7

*R = randomized patient; NR = nonrandomized patient.

REFERENCES

1. MARCINIAK TA, MOERTEL CG, SCHUTT AJ, ET AL. A phase II study of ICRF-159 (NSC-129943) in advanced colorectal carcinoma. Cancer Chemother Rep 59:761–763, 1975.
2. BALLET RE, ENGSTROM PF, CATALANO RB, ET AL. Phase II study of ICRF-159 in patients with metastatic colorectal carcinoma previously exposed to systemic chemotherapy. Cancer Treat Rep 60:1395–1397, 1976.
3. RODRIGUEZ V, RICHMAN SP, BENJAMIN RS, ET AL. Phase II study with Baker's antifol in solid tumors. Cancer Res 37:980–983, 1977.
4. MCCREARY RH, MOERTEL CG, SCHUTT AJ, ET AL. A phase II study of triazinate (NSC 139105) in advanced colorectal carcinoma. Cancer 40:9–13, 1977.
5. MOERTEL CG. Therapy of advanced gastrointestinal cancer with the nitrosoureas. Cancer Chemother Rep Part 3, vol 4(3):27–34, 1973.

Phase II Study of 5-FU, Doxorubicin, and Mitomycin (FAM) and Chlorozotocin in Advanced Measurable Pancreatic Cancer[1,2]

Frederick P. Smith,* Vinod K. Rustgi, Gerald Schertz, Paul V. Woolley, and Philip S. Schein[3]

The combination of 5-FU, doxorubicin, and mitomycin (FAM) has been demonstrated to be therapeutically active against advanced gastric and pancreatic carcinoma (1,2). For advanced pancreatic cancer, we have previously reported a 37% response rate, with a median response duration of 9 months (2). These results have been corroborated by Bitran et al (3), who obtained responses in five of 14 patients (40%) with pancreatic cancer, with a similar FAM regimen.

Chlorozotocin is a chloroethylnitrosourea with relative bone marrow-sparing properties compared to other compounds in its class (4); this allows it to be incorporated into drug combinations at near full dosage. We designed a phase II trial in which chlorozotocin was added to the FAM regimen to determine whether the combination could be tolerated and whether there were indications of enhanced therapeutic response.

MATERIALS AND METHODS

Between August 1978 and February 1980, 23 patients (13 females and ten males) with advanced pancreatic cancer were entered in the study. A histologically confirmed diagnosis of adenocarcinoma of the pancreas was required, and at the time of initiation of therapy all patients had extensive disease that was not amenable to surgical or radiotherapeutic management. No patient had been previously treated with chemotherapy or radiation therapy. All patients had objective measurable disease by either physical examination and/or special radiologic evaluations. The median age was 60 years (range, 41–76). The performance status (PS) of each patient was assessed at the time of entry in this study, using the Eastern Cooperative Oncology Group system. The median PS was 1, with a range of 0–4.

The FAM-chlorozotocin was administered in 8-week cycles. 5-FU (600 mg/m^2) was administered iv on Days 1, 8, 29, and 36; doxorubicin (30 mg/m^2), on Days 1 and 29; mitomycin (10 mg/m^2), on Day 1; and chlorozotocin (80 mg/m^2), on Day 29. Drug dosage was modified according to the degree of hematologic toxicity, which was measured by wbc and platelet counts as has been previously described for FAM (2).

Durations of response and survival were determined from initiation of FAM-chlorozotocin. A partial response was defined as a decrease of at least 30% in hepatomegaly or a $\geq$ 50% decrease, lasting at least 4 weeks, in the two largest perpendicular diameters of the most definable lesion, without progression of other disease or clinical deterioration. Stable disease was defined as a $<$ 50% reduction or a $<$ 25% increase in tumor size lasting at least 8 weeks.

RESULTS

Three of the 23 patients (13%) achieved a partial response. The median duration of response was 5.5 months (range, 2.5–6.5) from initiation of chemotherapy. Four patients achieved disease stabilization, while 15 had evidence of progressive tumor. The median survival for all patients is 6.4 months (range, 1–15).

FAM-chlorozotocin was well-tolerated. Myelosuppression was the only dose-limiting toxic effect. The median wbc count nadir was 4.0×10^3/mm^3 (range, 1.1–7.2), and platelet count nadir was 176×10^3/mm^3 (range, 90–500). There were no treatment-related deaths.

DISCUSSION

The FAM-chlorozotocin regimen employed in this phase II study does not have significant activity for

[1]Received Apr 22, 1982; revised Sept 14, 1982; accepted Sept 22, 1982.

[2]Supported by Public Health Service grant CA-25759 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Department of Medicine, Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Washington, DC.

**Reprint requests to:* Frederick P. Smith, MD, Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Rm 2100 W, 3800 Reservoir Rd, NW, Washington, DC 20007.

pancreatic cancer. In retrospective comparison to FAM, the addition of chlorozotocin appears to be equally well-tolerated; however, the response rate is lower (37% vs 13%, respectively), and the duration of response is shorter (9 vs 5.5 months, respectively). There were no apparent differences in PS or sites of disease between the groups to explain this discrepancy in responses. The chloroethylnitrosoureas do not have an established role in the management of pancreatic carcinoma. Past trial of carmustine demonstrated no responders among 21 patients, while semustine produced only an 8% response rate (5). Nonetheless, the reason for the discrepancy in responses and, more importantly, in durations of response between FAM and FAM-chlorozotocin is not elucidated by this study. We are not aware that chlorozotocin in other drug combinations has diminished the effectiveness of a standard regimen. However, based on these results we do not recommend further study of this FAM-chlorozotocin regimen for metastatic pancreatic cancer.

REFERENCES

1. MACDONALD JS, SCHEIN PS, WOOLLEY PV, ET AL. 5-Fluorouracil, doxorubicin, and mitomycin (FAM) combination chemotherapy for advanced gastric cancer. Ann Intern Med 93:533–536, 1980.
2. SMITH FP, HOTH DF, LEVIN B, ET AL. 5-Fluorouracil, Adriamycin and mitomycin-C (FAM) chemotherapy for advanced adenocarcinoma of the pancreas. Cancer 46:2014–2018, 1980.
3. BITRAN JD, DESSER RK, KOZLOFF MF, ET AL. Treatment of metastatic pancreatic and gastric adenocarcinomas with 5-fluorouracil, Adriamycin, and mitomycin C (FAM). Cancer Treat Rep 63:2049–2051, 1979.
4. HOTH DF, WOOLLEY PV, MACDONALD JS, ET AL. Phase I trial and clinical pharmacology. Clin Pharmacol Ther 23:712–722, 1978.
5. KOVACH JS, MOERTEL CG, SCHUTT AJ, ET AL. A controlled study of combined 1,3-bis-(2-chloroethyl)-1-nitrosourea for advanced gastric and pancreatic cancer. Cancer 33:563–567, 1974.

Phase II Trial of Aziridinylbenzoquinone (AZQ) in Head and Neck Cancer[1,2]

Arlene A. Forastiere,[3,*] Sidney M. Crain,[3] Kent Callahan,[4] David Van Echo,[3] Douglas Mattox,[5] Myo Thant,[6] Daniel D. Von Hoff,[4] and Peter H. Wiernik[3]

1,4-Cyclohexadiene-1,4-dicarbamic acid,2,5-bis(1-aziridinyl)-3,6-dioxodiethyl ester (AZQ) is one of a series of lypophilic aziridinylbenzoquinones that are known to cross-link DNA (1,2). In preclinical testing, AZQ demonstrated a broad spectrum of activity in murine tumor systems (3). In phase I testing, myelosuppression was dose-limiting; gastrointestinal toxicity and transient hepatic dysfunction were infrequent side effects (4). This study evaluated the activity of AZQ in head and neck cancer.

MATERIALS AND METHODS

Twenty-four patients with histologically confirmed epidermoid carcinoma of the head and neck were treated. All patients had measurable disease, a Karnofsky performance score ≥ 50, an expected survival ≥ 8 weeks, a wbc count $\geq 4000/\mu l$, a platelet count $\geq 100{,}000/\mu l$, a creatinine level ≤ 2.0 mg/dl, and a bilirubin level ≤ 1.5 mg/dl. Cbc and platelet counts were repeated weekly, and measurable disease parameters were assessed every 4 weeks.

AZQ was given at a dose of 20 mg/m^2 on Days 1 and 8 every 28 days. On Day 8 the dose was decreased for patients with grades 1, 2, and 3 toxic effects (Cancer and Leukemia Group B criteria) to 15, 10, and 7.5 mg/m^2, respectively. On Day 28 the dose was reduced to 15 mg/m^2 if grade 3 toxicity was reached during the previous cycle. An adequate trial of AZQ consisted of at least two cycles or rapid progression of disease after one cycle at a dose producing a wbc count nadir of $< 3000/\mu l$. Standard response criteria defined in previous trials were used (5).

RESULTS

Eighteen of 24 patients were evaluable for response. There were five early deaths after the first course (three from progressive disease, one from unknown causes, and one from sepsis secondary to drug-induced leukopenia), and one patient was lost to follow-up. Patient characteristics and toxicity to AZQ are detailed in table 1. All patients had had prior radiotherapy (RT), 71% had had surgery in addition to RT, and 66% had received prior chemotherapy. Disease recurred at distant sites in 50% of the patients.

Seventy-two percent of evaluable patients had at least two courses of AZQ. There was one minor response lasting 4 months in a patient with pulmonary metastases; all other patients had disease progression. Nineteen patients were evaluable for toxicity. Dose-limiting toxic effects were hematologic, resulting in dose reduction or treatment delay in six patients. There was one septic death and one life-threatening hemorrhage secondary to drug-induced thrombocytopenia. Hemoglobin dropped ≥ 2.0 g/dl in 12 patients (median, 2.7 g/dl; range, 2.0–6.2) from 1 to 8.5 weeks (median, 4) after the initial dose of AZQ. Progressive decline in hemoglobin was observed with repeated doses. There was no evidence of blood loss or hemolysis, and prior chemotherapy was not associated with increased toxicity.

DISCUSSION

No major responses were observed in this phase II trial of AZQ for advanced epidermoid carcinoma of the head and neck. The median performance status was 70

[1]Received May 17, 1982; revised Sept 16, 1982; accepted Sept 30, 1982.

[2]Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Medical Oncology Section, University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.

[4]Division of Oncology, Department of Medicine, The University of Texas Health Science Center at San Antonio.

[5]Division of Otorhinolaryngology, Department of Surgery, The University of Texas Health Science Center at San Antonio.

[6]Greater Baltimore Medical Center, MD.

**Reprint requests to:* Dr. Arlene A. Forastiere, VA Medical Center, 2215 Fuller Rd, Ann Arbor, MI 48105.

TABLE 1.—Patient characteristics and drug toxicity

Characteristics	No. of patients
Median age in yrs (range): 55 (22–85)	
Median performance status (range): 70 (50–80)	
Males/females	19/5
Prior treatment	
RT only	1
Surgery + RT	7
RT + chemotherapy	6
Surgery + RT + chemotherapy	10
Primary site	
Oral cavity	3
Oropharynx	10
Larynx	9
Pyriform sinus	2
Sites of measurable disease	
Primary	10
Regional lymph nodes	15
Skin	5
Lung	6
Liver	1
Courses of AZQ	
1	5
2	9
3	3
5	1
Toxicity	
Hematologic	
Wbc count nadir (cells/μl)	
3000–3999	1
2000–2999	4
< 2000	6
Platelet count nadir (cells/μl)	
50,000–100,000	3
< 50,000	3
Hemoglobin decrease (g/dl)	
2–4	9
> 4	3
Nausea	5
Vomiting	1
Stomatitis	1

and all patients except one had been previously treated with two or more treatment modalities. We conclude that in these heavily prior-treated patients, AZQ in this dose and schedule is not a useful agent.

REFERENCES

1. CHOU F, KAHN AH, and DRISCOLL JS. Potential central nervous system antitumor agents. Aziridinyl-benzoquinones 2. J Med Chem 19:1302–1308, 1976.
2. AKHTAR MH, BAGLEITER A, JOHNSON D, ET AL. Studies related to antitumor antibiotics. Part VI. Correlation of covalent cross-linking of DNA by bifunctional aziridinoquinones with their antineoplastic activities. Can J Chem 53:2891, 1975.
3. Clinical brochure. Aziridinylbenzoquinone (NSC-182986). Bethesda, MD, National Cancer Institute, Aug 1979.
4. SCHILSKY RL, KELLEY JA, IHDE DC, ET AL. Phase I trial and pharmacokinetics of aziridinylbenzoquinone (NSC 182986) in humans. Cancer Res 42:1582–1586, 1982.
5. FORASTIERE AA, YOUNG CW, and WITTES RE. A phase II trial of *m*-AMSA in head and neck cancer. Cancer Chemother Pharmacol 6:145–146, 1981.

Methyl-GAG in Advanced Colon Cancer: A Phase II Trial of the Southwest Oncology Group[1,2]

William A. Knight, III,[3] David M. Loesch,[3] Lawrence P. Leichman,[4] Carol Fabian,[5] and Robert M. O'Bryan[6,*]

Methyl-GAG is a compound with demonstrable activity in acute myelogenous leukemia (1) and lymphoma (2). Prohibitive toxicity with a daily schedule led to the use of an intermittent schedule by the Eastern Cooperative Oncology Group and the Eastern Drug Evaluation Program, resulting in decreased toxic effects (3). In a phase I–II pilot study (4), we utilized a weekly schedule of methyl-GAG at a starting dose of 500 mg/m^2. Acceptable toxic reactions and activity in patients with advanced colon cancer were the basis for this phase II trial.

PATIENTS AND METHODS

Forty patients with pathologically documented colorectal carcinoma and measurable disease were entered in the study. Of these, 36 had received no prior chemotherapy. Other characteristics included a median age of 55 years and a performance status of 1.2 (Southwest Oncology Group criteria).

Methyl-GAG was given as a weekly iv infusion in 100 ml of 5% dextrose in water for ≥ 45 minutes. The starting dose of 600 mg/m^2 was increased by 100 mg/m^2/week in the absence of toxic effects. A dose reduction of 100 mg/m^2 was required for grade 2 or 3 toxicity (Southwest Oncology Group criteria), and therapy was interrupted for grade 4 toxicity. Therapy was continued after all toxic effects resolved.

Complete response was defined as a complete regression of all measurable disease; partial response was defined as a > 50% reduction of the product of the two longest perpendicular diameters of the measurable lesions. If hepatomegaly was chosen as a followable parameter, a > 30% decrease in the sum of the measurements below the xiphoid process and both costal margins in the midclavicular line was designated as a partial remission. Patients were considered evaluable for response if they received two courses of methyl-GAG and were followed for at least 4 weeks.

RESULTS

Thirty-two patients were evaluable for response. Seven patients refused further therapy after one course, and one patient died of a myocardial infarction before assessment of measurable disease. Two patients, both with liver metastases, had partial responses of 53 and 29 weeks. Three patients had disease stablization for 43, 12, and 11 weeks.

Toxicity was evaluable for 40 patients and is summarized in table 1. Severe toxic reactions were seen in 14 patients, 13 with severe vomiting and one with hemorrhagic diarrhea. Such toxicity tended to occur after four weekly courses of treatment. Toxicity was reversible when the drug was withheld.

DISCUSSION

Methyl-GAG does not have significant activity among patients with metastatic colon cancer. Prolonged dis-

[1]Received July 19, 1982; accepted Sept 22, 1982.

[2]Supported in part by Public Health Service grants CA-22433, CA-14028, CA-12644, CA-04915, CA-12014, and CA-32102 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]University of Texas Health Sciences Center at San Antonio.

[4]Wayne State University, Detroit, MI.

[5]University of Kansas Medical Center, Kansas City.

[6]Henry Ford Hospital, Detroit, MI.

*_Reprint requests to:_ Southwest Oncology Group (SWOG-7940), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Toxic effects of methyl-GAG given weekly

Toxic effect	No. of patients
Nausea	26
Vomiting ≤ 6 times/day	7
Vomiting ≥ 7 times/day	6
Hospitalization secondary to vomiting	3
Anorexia	16
Diarrhea	14
3–4 stools/day, no dehydration	6
> 4 stools/day, iv hydration required	3
Bloody diarrhea	1
Mucositis	8
Ulcers, able to eat	3
Ulcers, unable to eat	5
Neuralgia	11
Circumoral paresthesia	13
Balanitis	2
Anemia	15
Decrease in Hgb ≥ 2 g/100 ml	9
Decrease in Hgb ≥ 3 g/100 ml	3
Decrease in Hgb ≥ 4 g/100 ml	3

ease stabilization, seen in a previous study (4), was not observed, and no explanation for this difference is apparent. Most patients in the former study had failed prior chemotherapy, possibly introducing a selection bias between the two studies. Toxicity in this study was more severe than in the previous study. Severe toxicity tended to be cumulative and might be averted by either a rest after four courses or a biweekly schedule. Based on these findings, further trials in colon cancer are not warranted.

REFERENCES

1. FREIREICH EJ, FREI E, and KARON M. Methylglyoxal bis(guanylhydrazone): a new agent active against acute myelocytic leukemia. Cancer Chemother Rep 16:183–186, 1962.
2. REGELSON W, and HOLLAND JF. Clinical experience with methylglyoxal bis(guanylhydrazone) dihydrochloride: a new agent with clinical activity in acute myelocytic leukemia and the lymphomas. Cancer Chemother Rep 27:15–26, 1963.
3. SHNIDER BI, COLSKY J, JONES R, ET AL. Effectiveness of methyl-GAG (NSC-32946) administered intramuscularly. Cancer Chemother Rep 58:689–695, 1974.
4. MYERS JW, KNIGHT WA, III, LIVINGSTON RB, ET AL. Phase I–II trial of methyl-GAG in advanced colon cancer. A Southwest Oncology Group pilot study. Cancer Clin Trials 4:277–279, 1981.

Compilation of 1982 *Clinical Trials Summaries* published in *Cancer Treatment Reports*

Tumor	Drug*	Dosage†	No. of evaluable patients	Response‡	Page
Advanced childhood cancers	Prednimustine	Regimen D: 40 mg/m²/day for ≥ 28 days Regimen E: 40 mg/m²/day for 14 days, then 60 mg/m²/day for ≥ 14 days Regimen I/200: 200 mg/m²/day × 5 q 14 days Regimen I/300: 300 mg/m²/day × 5 q 14 days	99 (all 4 regimens)	3 MR 1 PR (CML), 5 MR 1 PR (hepatoma), 1 MR 1 MR	1583
Breast	AMSA	90 or 120 mg/m² by 30-min infusion q 3–4 wks	27	2 MR	403
	AMSA + prednisone + chlorambucil	AMSA: 90 or 120 mg/m² iv on Day 1 q 22 days Prednisone: 40 mg/m²/day orally on Days 1–7 q 22 days Chlorambucil: 0.2 mg/kg orally on Days 1–7 q 22 days	30	NR	1235
	Aziridinylbenzoquinone	30 mg/m² by 30-min iv infusion q 3 wks	35	< 7 MR	1875
	Estracyt	560 mg/day orally in 2 divided doses	16	1 PR	581
	Gallium nitrate	700 mg/m² in 250 ml of NS over 30 mins following prehydration with 1500 ml of 5% dextrose in ½ NS q 2 wks	23	NR	1591
	Spirogermanium	80 mg/m² by 1-hr iv infusion on Monday, Wednesday, and Friday for 2 wks, then on Tuesday and Thursday for 2 wks, then once/wk	30	NR	1667
	Tamoxifen	20 mg twice/day (after disease progression during therapy) at 10 mg twice/day	30	NR	1445
	Vinblastine	1.2–1.8 mg/m²/day by continuous 5-day iv infusion q 3 wks	17	NR	1783
Colorectal	Bisantrene	260 mg/m² iv over ≥ 60 mins q 3 wks	32	NR	1997
	Cycloleucine	40–50 g/m² iv q 3 wks	29	NR	203
	Methyl-GAG	600 mg/m²/wk by ≥ 45-min iv infusion in 100 ml of D5W	32	2 PR	2099
	Mitoxantrone	8–12 mg/m² q 3 wks	45	NR	1995
		12 mg/m² by 30-min iv infusion in 150 ml of D5W q 3 wks	33	1 PR	1779
	Semustine + razoxane	Semustine: 150 mg/m² orally on Day 1 q 8 wks Razoxane: 500 mg/m² orally on Days 2–4 and 29–31 q 8 wks	27	1 PR	2093
	Semustine + Baker's antifol	Semustine: 150 mg/m² orally on Day 1 q 8 wks Baker's antifol: 200 mg/m² by 1-hr iv infusion on Days 1–3 and 29–31 q 8 wks	22	NR	2093
	Baker's antifol + razoxane	Baker's antifol: 125 mg/m² by 1-hr iv infusion on Days 1–3 and 29–31 q 8 wks Razoxane: 425 mg/m² orally on Days 1–3 and 29–31 q 8 wks	26	1 PR	2093
	Vindesine	2 mg/m² iv on Day 1 and 1 mg/m² iv on Day 2, q 7 or 10 days	15	NR	583
Endometrial carcinoma	Etoposide	100 mg/m² in 150 ml of NS by 45-min iv infusion on Days 1, 3, and 5 q 28 days	29	1 PR	1669
Head and neck					
Squamous cell carcinoma	Bleomycin + hydroxyurea	Bleomycin: 15 units/m² iv on Day 1 q 2 wks Hydroxyurea: 1000 mg/m²/day orally in divided doses on Days 8–15 q 2 wks	19	9 PR	1439
Epidermoid carcinoma	Aziridinylbenzoquinone	20 mg/m² on Days 1 and 8 q 28 days	18	1 MR	2097
	Thioproline	40–60 mg/kg/day × 5 q 3 wks	16	1 MR	585

Tumor	Drug*	Dosage†	No. of evaluable patients	Response‡	Page
Leukemia (adult)	AMSA + autologous bone marrow	AMSA: 800 mg/m^2 divided in 4 daily doses Bone marrow: 0.4–1.4 × 10^8 nucleated cells/kg	11	1 CR (AML) 1 PR (ALL) 1 MR (AML)	1991
Lung					
Bronchogenic carcinoma	BCNU + cyclophosphamide + doxorubicin	BCNU: 100 mg/m^2 iv on Day 1 Cyclophosphamide: 400 mg/m^2 iv on Day 1 Doxorubicin: 40 mg/m^2 iv on Day 2	29	1 CR (epidermoid) 1 PR (epidermoid)	199
	Methotrexate	0.6 mg/kg twice/wk	131	5%	197
	Cisplatin + methotrexate + citrovorum factor	Cisplatin: 60 mg/m^2 q 3 wks Methotrexate: 100 mg/m^2 q 3 wks Citrovorum factor: 5 mg/m^2	47	0%	197
	CCNU + cyclophosphamide + methotrexate	CCNU: 50 mg/m^2 orally q 6 wks Cyclophosphamide: 500 mg/m^2 iv q 3 wks Methotrexate: 10 mg/m^2 orally twice/wk	54	15%	197
	Cisplatin + CCNU + cyclophosphamide + methotrexate	Cisplatin: 75 mg/m^2 iv q 6 wks CCNU: 70 mg/m^2 q 6 wks Cyclophosphamide: 700 mg/m^2 q 3 wks Methotrexate: 15 mg/m^2 orally twice/wk	18	11%	197
Small cell	Mitomycin + hexamethylmelamine + vindesine	Mitomycin: 10 mg/m^2 iv on Day 1 q 43 days Hexamethylmelamine: 100 mg/day orally on Days 1–14 and 21–36 q 43 days Vindesine: 3 mg/m^2 iv on Days 1, 8, 21, and 28 q 43 days	13	1 PR	1873
Squamous cell	Vincristine + BCNU + bleomycin	Vincristine: 1.2 mg/m^2 iv at 0 hrs q 2 wks BCNU: 100 mg/m^2 iv at 0 hrs q 2 wks Bleomycin: 5 mg im at 6, 30, 54, 78, and 102 hrs q 6 wks	27	2 MR	1877
Unspecified	DON	160 mg/m^2/day iv × 3 q 3 wks	22	NR	1665
Lymphoma (non-Hodgkin's)	Maytansine	0.5 or 0.75 mg/m^2/wk iv	17	1 PR	1587
Melanoma	AMSA	150 mg/m^2 iv q 21 days	16	NR	1237
	Megestrol acetate	250 mg twice/day orally	20	NR	1239
	Mitolactol	180 mg/m^2/day orally × 10 q 42 days	20	NR	195
	Tamoxifen	10 mg twice/day	23	NR	1771
		10 mg twice/day orally	18	1 PR	1447
		40 mg/day orally in 2 divided doses	21	1 PR	1437
Ovarian	AMSA	90 or 120 mg/m^2 iv over 30 mins q 3 wks	20	NR	1589
	Hexamethylmelamine (or cisplatin) + cyclophosphamide + methotrexate + 5-FU	Hexamethylmelamine: 150 mg/m^2/day orally on Days 1–14 q 4 wks (Cisplatin: 50 mg/m^2 iv on Day 1) Cyclophosphamide: 100 mg/day orally on Days 1–14 q 4 wks Methotrexate: 25 mg/m^2 iv on Days 1 and 8 q 4 wks 5-FU: 300 mg/m^2 on Days 1 and 8	18 (35)	1 PR (5 PR)	1233
	Medroxyprogesterone acetate	500 mg/m^2/day orally in 3 divided doses 500 mg/day im for 3 wks, then 500 mg once/wk for ≥ 2 mos	27 25	1 PR 1 PR	407 1441
	Spirogermanium	60 mg/m^2 iv 3 ×/wk during Wks 1 and 2, 60–80 mg/m^2 2 ×/wk during Wks 3 and 4, 60–80 mg/m^2 1 ×/wk at Wk 5 and beyond	13	NR	1675
	Yoshi 864	2.0 mg/kg/day × 5 by slow iv push q 6 wks	33	NR	1775

published in *Cancer Treatment Reports* (continued)

Tumor	Drug*	Dosage†	No. of evaluable patients	Response‡	Page
Pancreatic adenocarcinoma	5-FU + doxorubicin + mitomycin + chlorozotocin	5-FU: 600 mg/m^2 iv on Days 1, 8, 29, and 36 q 8 wks Doxorubicin: 30 mg/m^2 on Days 1 and 29 q 8 wks Mitomycin: 10 mg/m^2 on Day 1 q 8 wks Chlorozotocin: 80 mg/m^2 on Day 29 q 8 wks	23	3 PR	2095
Prostatic carcinoma	*m*-AMSA	100 or 120 mg/m^2 q 3 wks	21	NR	1993
Renal	Dianhydrogalactitol	15, 25, or 30 mg/m^2/day iv × 5 q 4 wks	41	1 PR	1231
	Tamoxifen + medroxyprogesterone acetate	Tamoxifen: 10 mg orally 3 ×/day Medroxyprogesterone acetate: 1.0 g/wk im for ≥ 4 wks	14	NR	1773
	Vinblastine + methyl-GAG	Vinblastine: 5 mg/m^2 by bolus iv infusion on Day 1 q 21 days Methyl-GAG: 500 mg/m^2 by bolus iv infusion on Days 2 and 8 q 21 days	15	NR	1585
	Yoshi 864 + medroxyprogesterone acetate	Yoshi 864: 60 or 80 mg/m^2/day × 5 by iv push q 49 days Medroxyprogesterone acetate: 40 mg/wk im	30	2 PR	1781
Transitional cell carcinoma of urinary tract	VM-26	20 or 30 mg/m^2/day × 5 by 1-hr infusion in 100 ml of NS q 3 wks	40	2 PR	405
Sarcoma	Azapicyl	350 mg/m^2/day orally × 14, 400 mg/m^2/day at Day 14, 450 mg/m^2/day at Day 28	22	NR	399
	Gallium nitrate	700 mg/m^2 by 30-min iv infusion q 2 wks	31	NR	1673
	Prednimustine	25 mg/m^2/day orally	25	1 CR (hemangiopericytoma) 2 PR (fibrohistiocytoma, leiomyosarcoma)	201

*BCNU = carmustine, CCNU = lomustine, VM-26 = teniposide.

†NS = normal saline, D5W = 5% dextrose in water.

‡NR = no response, MR = minor response, PR = partial response, CR = complete response, ALL = acute lymphocytic leukemia, AML = acute myelocytic leukemia, CML = chronic myelogenous leukemia.

Aziridinylbenzoquinone (AZQ) Treatment of Central Nervous System Leukemia

Preclinical and clinical studies have shown that aziridinylbenzoquinone (AZQ) crosses the blood-brain barrier and has antitumor activity against some brain tumors (1–4). Recently we have completed a phase I evaluation of AZQ in pediatric patients with refractory acute leukemia. Doses ranged from 5 to 36 mg/m^2 daily × 5. In this and other studies, AZQ demonstrated antileukemic action (5). Our study included two patients with both bone marrow and CNS leukemia. Both patients showed improvement in the CNS leukemia following iv administration of AZQ.

CASE REPORTS

Patient 1

A 3$^1/_2$-year-old white female was diagnosed as having common cell acute lymphocytic leukemia in September 1980. The presenting wbc count was 55,000/mm^3. After achieving a remission with standard chemotherapy, she received intrathecal methotrexate and cranial irradiation. Maintenance therapy was 6-mercaptopurine and methotrexate plus 3-day courses of cytarabine and cyclophosphamide every 8 weeks. The patient presented with a CNS relapse approximately 4 months after the cranial irradiation. She had a seizure after the first intrathecal dose of methotrexate and was therefore treated with intrathecal cytarabine and craniospinal irradiation. A bone marrow relapse occurred in October 1981. Treatment with vincristine, prednisone, L-asparaginase, and doxorubicin for 4 weeks failed to produce remission, so the patient was started on teniposide and cytarabine. Two weeks later a spinal tap revealed 1630 cells/mm^3, all blasts. A cbc showed a hemoglobin of 7.8 g/dl; a wbc count of 4200/mm^3, with 3% blasts; and a platelet count of 12,000/mm^3. Bone marrow was M3. The patient was started on AZQ, 24 mg/m^2 daily × 5. One hour after her fifth dose of AZQ, a repeat spinal tap showed only 14 reactive cells/mm^3. The AZQ concentrations in cerebrospinal fluid and in plasma were 120 ng/ml and 200 ng/ml, respectively (courtesy of Dr. John S. Kovach, Mayo Clinic). At that time, hemoglobin was 7.0 g/dl; platelet count, 78,000/mm^3; and wbc count, 900/mm^3 with 1% blasts. Approximately 4 weeks later the patient again developed lethargy and bizarre behavior. A repeat spinal tap revealed 530 lymphoblasts/mm^3. The peripheral blood was still pancytopenic, and a bone marrow sample was hypocellular with residual leukemic cells. A second course of AZQ was given at the same dose. One week after the last dose, the spinal fluid was clear of leukemic cells but was slightly xanthochromic. The patient remained pancytopenic, developed gastrointestinal bleeding and hepatomegaly, and died of gram-negative sepsis 2 weeks later. Post-mortem examination revealed a hypoplastic bone marrow with residual leukemic cells. Focal leukemic infiltrates were seen in the liver and the kidney. Cytocentrifuge preparations of the cerebrospinal fluid contained no lymphoblasts. Brain pathology was not investigated.

Patient 2

A white male, 2 years 10 months old, was diagnosed as having acute lymphocytic leukemia in January 1975. He was started on AZQ in October 1980 after developing a third CNS relapse. Prior pertinent history included testicular disease treated with 2000 rads; bilateral parotid gland infiltrates, also treated with radiation; and five prior bone marrow relapses between March 1978 and October 1980. AZQ (6 mg/m^2 daily × 5) was given during the last week of October 1980. A spinal tap immediately prior to the first dose of AZQ revealed a wbc count of 470/mm^3 with blasts. A repeat spinal tap on the last day of therapy revealed a wbc count of 27/mm^3, with few residual blasts. Neurologic examination was normal. The initial peripheral wbc count was 13,800/mm^3 with 76% blasts. On the last day of therapy the wbc count was 9000/mm^3 with 90% blasts. Two weeks after AZQ the peripheral wbc count was 700/mm^3 with 40% blasts, and a spinal tap showed 240 cells/mm^3. The patient then received intrathecal methotrexate. Because of persistent marrow disease the patient received a second course of AZQ approximately 1 week later. Two weeks after the last dose of AZQ, he died at home of sepsis; he was pancytopenic with residual leukemia. No autopsy was performed.

DISCUSSION

This report demonstrates that systemic AZQ can lower lymphoblast counts in the cerebrospinal fluid. In the first case, response occurred in a patient who had seizures after methotrexate and had recurrences after 5400 rads to the cranium, 1800 rads to the spinal column, and intrathecal cytarabine. The second case also documented a transient response of CNS leukemia in a

patient who had had two prior CNS relapses and five bone marrow relapses. In both cases the AZQ produced no signs of CNS toxicity.

Although the incidence of CNS leukemia has been dramatically reduced in children with acute leukemia by the use of CNS prophylaxis, it is still a most difficult problem when it occurs, because the current useful drugs usually fail to eradicate this site of disease and are often associated with severe CNS toxicity. The responses we have seen suggest that AZQ may be a new treatment for CNS leukemia. In these patients, CNS relapse was also associated with bone marrow disease, and therefore, the schedule was designed to treat refractory bone marrow leukemia. A schedule to treat isolated CNS disease has not yet been established. The ability to transiently treat CNS leukemia at even low doses of AZQ warrants more studies and trials of this agent in the treatment of CNS disease.

REFERENCES

1. Gormley PE, Wood JH, and Poplack DG. Ability of a new antitumor agent, AZQ, to penetrate into cerebrospinal fluid. Pharmacology 22:196–198, 1981.
2. Curt GA, Schilsky R, Kelly J, et al. Phase II study of aziridinylbenzoquinone in high grade gliomas. Proc ASCO 1:13, 1982.
3. Aroney RS, Kaplan RS, Salcman M, et al. A phase II trial of AZQ in patients with recurrent primary or metastatic brain tumor. Proc ASCO 1:24, 1982.
4. Bachur NR, Collins JM, Kelley JA, et al. Diaziquone, 2,5-diaziridinyl-3-6-biscarboethoxyamino-1,4-benzoquinone, plasma and cerebrospinal fluid kinetics. Clin Pharmacol Ther 31:650–655, 1982.
5. Tan C, Hancock C, Miller L, et al. Phase I study of aziridinylbenzoquinone in children with cancer. Proc Am Assoc Cancer Res 23:120, 1982.

Barton A. Kamen
John S. Holcenberg

Midwest Childrens Cancer Center
Milwaukee Childrens Hospital and
Medical College of Wisconsin
1700 W Wisconsin Ave
Milwaukee, WI 53233

Stuart E. Siegel

Childrens Hospital of Los Angeles
Los Angeles, CA 90054

July 6, 1982

Apparent Cyclophosphamide Hypersensitivity: Urticaria Associated With Cyclophosphamide Without Prior Sensitization

Urticaria reaction to cyclophosphamide is so rare that it was not mentioned in recent detailed articles on skin reactions due to chemotherapy (1,2). To date, there are nine reported cases of anaphylactic and urticarial reactions due to iv cyclophosphamide (Cytoxan) (3,4). The triggers of such reactions are not exactly known, and experience with cross-sensitivity to other alkylating agents is not uniform (5,6). With only one exception (7), all previously reported cases either occurred after several weeks or years of treatment with cyclophosphamide or patients had experienced hypersensitivity to other alkylating agents. The present case is unique in that there was no prior exposure to any chemotherapy.

CASE REPORT

A 56-year-old male was diagnosed as having a mucin-secreting adenocarcinoma of the lung in February 1978. He had diffused bony metastasis. Between March 14 and May 8 he received 6000 rads to the lumbar spine to relieve severe pain. He first received chemotherapy on May 30, 1978. The medications consisted of iv cyclophosphamide, doxorubicin, and methotrexate and im prochlorperazine (Compazine). A few minutes later the patient developed a generalized urticaria reaction. He responded promptly to iv diphenhydramine hydrochloride (Benadryl) and methylprednisolone (Solu-Medrol) and was discharged on the same day in good condition. On June 8 and July 3, 1978, the patient received only doxorubicin and methotrexate and, subsequently, doxorubicin, methotrexate, carmustine, and prochlorperazine every 3 weeks for 2 months, without experiencing urticaria reactions.

This is an unusual case in which there was no prior exposure to cyclophosphamide. Urticaria reaction occurred during the first dose of the drug.

It is conjectural whether radiation, which has alkylating properties, could be the sensitizing agent. However, carmustine, which also has alkylating properties, did not produce any hypersensitization effect in our patient or that of Ross and Chabner (5). This experience is also in accord with that of Legha and Hall (6), who found no cross-sensitization between cyclophosphamide, ifosfamide, and mechlorethamine. After a careful study, Lakin and Cahill (8) concluded that cyclophosphamide reaction was mediated by IgE antibody in their patient. No other detailed study has been done to define clearly the mechanism of cyclophosphamide sensitivity. The exact mechanism of cyclophosphamide sensitivity in our patient remains an enigma, since there was no prior exposure to chemotherapy.

REFERENCES

1. LEVINE ML, and GREENWALD ES. Mucocutaneous side effects of cancer chemotherapy. Cancer Treat Rev 5:67–84, 1978.
2. ADRIAN RM, HOOD AF, and SKARIN AT. Mucocutaneous reactions to antineoplastic agents. CA 30:143–157, 1980.
3. WEISS RB, and BRENO S. Hypersensitivity reactions to cancer chemotherapeutic agents. Ann Intern Med 94:66–72, 1981.
4. WEISS RB. Hypersensitivity reactions to cancer chemotherapy. Semin Oncol 9:5–13, 1982.
5. ROSS WE, and CHABNER BA. Allergic reactions to cyclophosphamide in a mechlorethamine-sensitive patient. Cancer Treat Rep 61:495–496, 1977.
6. LEGHA SS, and HALL S. Acute cyclophosphamide hypersensitivity reactions: possible lack of cross-sensitivity to mechlorethamine and isophosphamide. Cancer Treat Rep 62:180–181, 1978.
7. KRUTCHIK AN, BUZDAR AU, and TASHIMA CK. Cyclophosphamide-induced urticaria. Occurrence in a patient with no cross-sensitivity to chlorambucil. Arch Intern Med 138:1725–1726, 1978.
8. LAKIN JD, and CAHILL RA. Generalized urticaria to cyclophosphamide: type I hypersensitivity to an immunosuppressive agent. J Allergy Clin Immunol 58:160–171, 1976.

Vincent Anku[1]

Adult Hematology-Oncology
St. John & Westshore Hospital
Campus Medical Arts Bldg #370
29101 Health Campus Dr
Westlake, OH 44145

August 20, 1982

[1]I am grateful to Dr. John W. Harris for his critique and valuable suggestions.

High-Dose Metoclopramide in Cancer Chemotherapy-Induced Nausea and Vomiting

Nausea and vomiting are among the more frequent and debilitating side effects of cancer chemotherapy. The control of nausea and vomiting induced by highly emetic drugs like cisplatin is known to be problematic (1). Even though conventional doses of metoclopramide have produced controversial effects (1,2), high doses have been found to be well-tolerated and effective (3,4). The preliminary results of a phase II study we have initiated indicate that it is unnecessary to use high doses for every patient and that high-dose metoclopramide can induce more side effects than reported by Gralla et al (3,4), especially in relatively young patients.

There were 33 men and 36 women in the study; all were adults (median age, 55 years). Chemotherapy regimens included cisplatin (75–120 mg/m^2) for 50 patients, dacarbazine (250–400 mg/m^2/day) for 16 patients, and dactinomycin (15 μg/kg/day) for three patients. All these agents can be considered to be highly emetic. Thirty patients had already received one or more courses of chemotherapy. High-dose metoclopramide (HDM) was at first given at a dose of 2 mg/kg in two 1-mg/kg doses. The first dose was an iv infusion over a period of 15 minutes, given 30 minutes before chemotherapy, and the second was given either as a pulse injection 15 minutes after chemotherapy or as a 1- or 4-hour infusion during chemotherapy.

Nineteen patients (27.5%) had no nausea or vomiting, 27 (39.1%) had nausea with perhaps one short episode of vomiting, and ten (14.5%) vomited less than five times over a period of < 3 hours. There was no effect in eight patients (11.6%), and in five patients the effectiveness of HDM was unclear. The antiemetic effect was seen regardless of the chemotherapy used, although it was not as complete when energetic combinations were used (5-FU plus doxorubicin and cisplatin or high-dose cisplatin plus high-dose cyclophosphamide and vindesine). Of the 30 patients who had already received one or more courses of the same chemotherapy, 22 showed a marked antiemetic effect of HDM. Four of 34 patients who received several treatments of chemotherapy with HDM had slight but progressive digestive problems.

Undesirable effects of HDM were seen in 25 patients (36.3%) and consisted of trismus (13 patients); diarrhea (ten); dyskinesia, especially ocular dyskinesia (eight); agitation and anxiety (seven); and drowsiness (three). These effects were considered to be minor in nine patients and troublesome in eight; they required HDM to be stopped in eight of the 25 patients. These effects were always noticed after the first treatment. In four of the eight patients for whom HDM treatment was discontinued, a dose increase to 3–4 mg/kg had been tried. Important side effects of HDM were seen in eight of nine patients younger than age 30.

HDM is a good antiemetic with marked effectiveness for two-thirds of the patients in our series, all of whom received highly emetic chemotherapy. It failed in only 11.6% of the patients. Three points are worth making. First, our results are comparable to those of Gralla et al (3,4), while using one-fifth of the dose of metoclopramide. Second, this decreased dose reduces the administration time of HDM, which makes it compatible with outpatient chemotherapy. Third, even though our doses were much smaller, undesirable effects of HDM were seen in one-third of our patients, which is different from the results of Gralla et al (3,4). Toxic effects were encountered with an unexpected high frequency in young

patients, 18–30 years old, who were not considered in the Gralla studies. We therefore feel that further studies are needed, particularly with respect to metoclopramide toxicity, before HDM can be recommended for general use.

REFERENCES

1. Poster DS, Penta JS, and Bruno S. Treatment of Cancer Chemotherapy Induced Nausea and Vomiting. New York, Masson, 1981.
2. Kahn T, Elias EG, and Mason GR. A single dose of metoclopramide in the control of vomiting from *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 62:1106–1107, 1978.
3. Gralla RJ, Itri LM, Pisko SE, et al. Antiemetic efficacy of high-dose metoclopramide: randomised trials with placebo and prochlorperazine in patients with chemotherapy induced nausea and vomiting. N Engl J Med 305:905–909, 1981.
4. Gralla RJ, Braun TJ, Squillante A, et al. Metoclopramide: Initial Clinical Studies of High Dosage Regimens in Cisplatin And Vomiting (Poster DS, Penta JS, and Bruno S, eds). New York, Masson, 1981, pp 167–175.

N. B. Bui
G. Marit
B. Hoerni

Fondation Bergonié
F 33076 Bordeaux, France

September 13, 1982

High-Dose Metoclopramide: Control of Dacarbazine-Induced Emesis in a Preliminary Trial[1]

Metoclopramide at high doses given iv has been found to be a safe and effective antiemetic agent (1–4). Double-blind, randomized trials have shown this agent to be superior to placebo (1), prochlorperazine (1), and tetrahydrocannabinol (2) in patients given cisplatin. The following open study was initiated to test the efficacy of metoclopramide in patients receiving dacarbazine, another agent likely to produce emesis.

To date, ten patients receiving dacarbazine at a dose of 1000 mg/m^2 iv over 60–90 minutes have been entered in this study. Patient characteristics included: a median age of 55 years (range, 30–66); a median performance status of 70% (range, 60%–90%); and a diagnosis of malignant melanoma in eight patients, of pancreatic islet cell carcinoma in one, and of soft tissue sarcoma in one. Seven patients had not previously received chemotherapy. The metoclopramide dose of 2.0 mg/kg was given iv over 15 minutes beginning 30 minutes before dacarbazine and was continued every 2 hours for four doses. All patients were observed in the hospital over a 24-hour period and were given no other chemotherapy, with the exception of one patient who also received dactinomycin at a dose of 1.5 mg/m^2 iv.

Nine of the ten patients had major antiemetic responses (0–2 emetic episodes), with four patients having no nausea or vomiting (including the patient receiving dactinomycin with dacarbazine). With this number of patients, the predicted true major antiemetic response rate would be 60%–90% ($P = 0.05$). One patient had a minor antiemetic response (3–5 emetic episodes). The median number of emetic episodes was one (range, zero to five episodes). Only mild side effects occurred, including mild sedation in seven patients and diarrhea (more than three loose bowel movements over 24 hours) in one patient. No extrapyramidal reactions were observed.

The patients entered in this study were all given dacarbazine at a high dose, which generally causes severe emesis (5). With the encouraging results observed in this small number of patients, it appears that larger scale trials with high doses of metoclopramide are indicated in patients receiving dacarbazine.

REFERENCES

1. Gralla RJ, Itri LM, Pisko SE, et al. Antiemetic efficacy of high dose metoclopramide: randomized trials with placebo and prochlorperazine in patients with chemotherapy induced vomiting. N Engl J Med 305:905–909, 1981.
2. Gralla RJ, Tyson LB, Clark RA, et al. Antiemetic trials with high dose metoclopramide: superiority over THC, and preservation of efficacy in subsequent chemotherapy courses. Proc ASCO 1:58, 1982.
3. Daniels M, and Belt J. High dose metoclopramide as an antiemetic for patients receiving chemotherapy with cisplatin. Oncol Nurs Forum 9:20–22, 1982.
4. Strum SB, McDermed JE, Opfell RW, et al. Intravenous metoclopramide: an effective antiemetic in cancer chemotherapy. JAMA 247:2683–2686, 1982.
5. Costanzi JJ. DTIC (NSC-45388) studies in the Southwest Oncology Group. Cancer Treat Rep 60:189–192, 1976.

Leslie B. Tyson
Rebecca A. Clark
Richard J. Gralla*

Developmental Chemotherapy and Solid Tumor Services
Department of Medicine
Memorial Sloan-Kettering Cancer Center
Cornell University Medical College
1275 York Ave
New York, NY 10021

September 20, 1982

[1]Supported in part by a grant from the A. H. Robins Co, Public Health Service grant CA-05826, and contract N01-CM-07337 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

*To whom reprint requests should be sent.

Subject Index

A

ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine)
cyclic delivery of MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) and, in stage IV Hodgkin's disease, 881
Acivicin
plasma and cerebrospinal fluid pharmacokinetics of, in Ommaya reservoir-bearing rhesus monkeys, 1333
Aclacinomycin A
flare (skin reaction), 1683
a phase I trial of, 1127
ACNU (1-(aminomethyl)-3-(2-chloroethyl)-3-nitrosourea)
with 5-FU and mitomycin (FUM) for carcinoma of the pancreas, 1645
Actinomycin D *see* Dactinomycin
Adenosine
comparision of the effects on cultured L1210 leukemia cells of the ribosyl, 2′-deoxyribosyl, and xylosyl homologs and tubercidin and, alone or combined with 2′-deoxycoformycin, 317
Adriamycin *see* Doxorubicin
Adverse effects, animals
cardiac and renal toxicity of daunorubicin compared in the rat and rabbit, 395
cisplatin-induced emesis in the ferret: a new animal model, 187
cyclophosphamide-induced cardiomyopathy in the rat, 1521
ganglioneuropathy in rabbits and rhesus monkey due to high cumulative doses of doxorubicin, 1349
malnutrition effect on methotrexate toxicity and tissue levels of dihydrofolate reductase in the rat, 85
noninvasive documentation of emesis in cats, 362
prevention of doxorubicin-induced skin ulcers in the rat and pig with dimethyl sulfoxide (DMSO), 1371
toxicity of aziridinylbenzoquinone administered iv to beagle dogs, 1845
toxicity (local and systemic) resulting from large-volume ip administration in the rat, 157
vomiting induced by cyclophosphamide and phosphoramide mustard in cats, 1625
Adverse effects, man
aclacinomycin A flare (skin reaction), 1683
acute ECG changes associated with AMSA treatment, 1678
acute leukemia after semustine (methyl-CCNU) therapy: a case report, 1593
acute vascular episodes associated with therapy for carcinomas of the upper aerodigestive tract with bleomycin, vincristine, and cisplatin, 369
allergic reaction following administration of AMSA, 594
aminoglutethimide-induced thrombocytopenia, 1785
antidiuretic hormone (ADH) elevation with chemotherapy-induced emesis, 25
anticipatory vomiting in women receiving cyclophosphamide, methotrexate, and 5-FU (CMF) adjuvant chemotherapy for breast carcinoma, 1601
apparent cyclophosphamide sensitivity: urticaria associated with cyclophosphamide without prior sensitization, 2106
cardiac abnormalities after AMSA administration, 483
combination antiemetics, 1449
the course of nausea and vomiting after high-dose cyclophosphamide, 1487
effective control of cisplatin-induced nausea using high-dose steroids and droperidol, 243
enhanced bleomycin toxicity during acute renal failure, 592
epileptic seizures associated with cisplatin administration, 1719
fatal hepatotoxicity associated with AMSA therapy, 1863
fever due to 6-mercaptopurine, 1785
hemolytic-uremic syndrome associated with mitomycin therapy, 457
inadvertent overdose with lomustine (CCNU) followed by hematologic recovery, 1241
leukopenia associated with aminoglutethimide therapy: a case report, 1879
leukemia occurring after treatment of small cell lung cancer, 1683
mitomycin-induced hemolytic uremic syndrome: case presentation and review of literature, 1244
nasal cartilage necrosis following high-dose 5-FU: a case report, 1884
neurologic and cardiac toxicity following iv application of methotrexate, 1561
ototoxicity in patients receiving cisplatin: importance of dose and method of drug administration, 19
persistent hypomagnesemia following cisplatin chemotherapy for testicular cancer, 1767
potential cardiotoxicity with mitoxantrone, 1641
pulmonary fibrosis associated with lomustine (CCNU): a case report, 1881
reactions to AMSA in medical personnel, 1885
severe toxicity and death associated with zinostatin administration, 410
streptozocin-induced eosinophilia and fever: a case report, 1449
subcutaneous reaction due to intra-arterial infusion of doxorubicin, 2000
unusual reaction to VP-16-213 and avoidance by prolonged infusion, 409
vindesine-associated angina and ECG changes, 587
vindesine overdose, 1247
L-Alanosine
using a daily × 3 schedule: a phase I study, 73
Alkeran *see* Melphalan
Allopurinol
cerebrospinal fluid versus serum concentrations of 5-FU, oxypurinol and, during treatment of metastatic brain can-

cer with 5-FU infusion, allopurinol, and radiation, 571
combined with 5-FU and hypoxanthine in chemotherapy for human colon adenocarcinoma xenografts, 1201
and 5-FU: toxicity modulation and phase II results in colon cancer, 1133
modulation of 5-FU toxicity with high dose of: phase I trial of an outpatient dose schedule, 1723
Aminoglutethimide
leukopenia associated with therapy, 1879
thrombocytopenia induced by, 1785
AMSA
acute ECG changes associated with treatment, 1678
adverse reactions to, in medical personnel, 1885
allergic reaction following administration of, 594
autologous bone marrow transplantation and high-dose, in relapsed acute leukemia, 1991
cardiac abnormalities after administration of, 483
evaluation in patients with stage III–IV malignant melanoma: a phase II study, 1237
fatal hepatotoxicity associated with therapy, 1863
high-dose, and bone marrow rescue in patients with solid tumors, 385
in patients with advanced ovarian cancer: a phase II study, 1242
in patients with metastatic breast cancer: a Cancer and Leukemia Group B phase II study, 1211
with prednisone and chlorambucil (APC) in the treatment of refractory breast cancer: a Northern California Oncology Group study, 1235
in previously treated patients with stage III and IV ovarian cancer: a phase II study, 1589
in prostatic cancer: a phase II study, 208
promising new agent in refractory acute leukemia, 1475
therapy for children with lymphoblastic malignancy, 593
trial of, in acute leukemia, 1596
in vitro evaluation of the cardiotoxic potential of, 1571
***m*-AMSA**
administered as a continuous infusion: a phase I–II study, 1813
in disseminated prostatic carcinoma: a phase II study, 1993
in hepatocellular carcinoma: a Southwest Oncology Group phase II study, 1651
Anal cancer *see* Cancer, anal
Anguidine
in gastrointestinal malignancies: a Southwest Oncology Group phase II study, 381
Anthracenedione
response of transplantable tumors of mice to derivatives alone and in combination with clinically useful agents, 1187
Anthracyclines
lack of correlation between effects of, on in vitro cardiac guanyl cyclase and in vivo cardiotoxicity, 1677
Antidiuretic hormone (ADH)
elevation associated with chemotherapy-induced emesis in man, 25
Antineoplastic drugs
absorption following large-volume ip administration to rats, 147
9-β-D-Arabinofuranosyladenine (Ara-A)
and 2′-deoxycoformycin (DCF) in the treatment of refractory acute myelocytic leukemia, 253
Ara-C *see* Cytarabine
Aryldimethyltriazenes
metabolism and mechanism of the antileukemic action of isomeric, 1751
prophylactic antimetastatic treatment as adjuvants to surgical tumor removal in mice bearing Lewis lung carcinoma, 115
L-Asparaginase
randomized phase II comparison of methotrexate with or without, in non-small cell cancer of the lung, 541
5-Azacitidine
treatment of the blast crisis of chronic myelogenous leukemia with VP-16-213 (etoposide) and, 267
and zorubicin for patients with previously treated acute nonlymphocytic leukemia: a Cancer and Leukemia Group B pilot study, 563
Azapicyl
evaluation in soft tissue sarcomas in children: a report from the Children's Cancer Study Group, 399
Aziridinylbenzoquinone (AZQ)
in advanced breast cancer: a Cancer and Leukemia Group B phase II trial, 1875
biochemical activation to its free radical species, 339
clinical and pharmacologic studies of, 1321
in disseminated malignant melanoma: a phase II study, 2089
in head and neck cancer: a phase II trial, 2097
toxicity of, administered iv to beagle dogs, 1845
in the treatment of central nervous system leukemia, 2105
AZQ *see* Aziridinylbenzoquinone

B

Baker's antifol
studies of methotrexate, razoxane, and, in advanced gastric cancer: a Gastrointestinal Tumor Study Group report, 1713
BCNU *see* Carmustine
Benzohydroxamic acids
cytotoxic and cell kinetic effects of 3,4,5-trihydroxybenzohydroxamic acid, 2071
modulation of cytarabine metabolism in the human promyelocytic leukemia cell line HL-60 by polyhydroxy-substituted, 1825
Bisantrene
in advanced colorectal cancer: a Cancer and Leukemia Group B phase II study, 1997
for advanced hypernephroma: a phase II trial, 1653
disposition and metabolic profile of a new antitumor agent in laboratory animals, 1173
in patients with renal cell carcinoma: a phase II evaluation, 1869
a phase I clinical evaluation of, 1517
Bleomycin
acute vascular episodes associated with therapy for carcinomas of the upper aerodigestive tract with vincristine, cisplatin, and, 369
with cisplatin, vincristine, and methotrexate in advanced non-small cell bronchogenic carcinoma, 205
combined chemotherapy with doxorubicin, vinblastine, and dacarbazine, and radiotherapy for advanced lymphoepithelioma, 1207

combination with vinblastine and cisplatin in advanced malignant melanoma: a phase II study, 567
coronary artery disease in presence of therapy with, 410
with cyclophosphamide, doxorubicin, vincristine, and prednisone, compared with doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine in the treatment of diffuse histiocytic lymphoma, 1279
enhanced toxicity of, during acute renal failure, 592
and hydroxyurea combination chemotherapy in the treatment of advanced head and neck cancer, 1439
intracavitary, in the management of malignant effusions: a multicenter study, 1903
with mitomycin, sequential combination in advanced cervical cancer: a Northern California Oncology Group study, 163
potentiation of toxicity by oxygen, 359
serial analysis of melanoma growth kinetics in a patient receiving procarbazine and: comparison of tumor volume, flow cytometry, and thymidine labeling techniques, 529
with vincristine and carmustine (BCNU) in patients with squamous cell bronchial carcinoma resistant to cyclophosphamide, doxorubicin, methotrexate, and procarbazine (CAMP), 1877
with vinblastine and cisplatin, treatment of advanced malignant melanoma with, 1299
Brain cancer *see* Cancer, brain
Breast cancer *see* Cancer, breast
Bronchial cancer *see* Cancer, bronchial
Bronchogenic cancer *see* Cancer, bronchogenic
Butorphanol
antiemetic activity against cisplatin-induced emesis in ferrets and dogs, 1831

C

CAM (cyclophosphamide, doxorubicin, and methotrexate)
random prospective study of combination chemotherapy versus single-agent sequential chemotherapy in non-small cell lung cancer, 2005
CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine)
combination chemotherapy with vincristine, carmustine (BCNU), and bleomycin in patients with squamous cell bronchial carcinoma resistant to, 1877
Cancer
advanced
indicine-*N*-oxide in patients with: a phase I study, 1509
pilot study of PALA (*N*-phosphonoacetyl-L-aspartate) and 5-FU in patients with, 77
anal
combined radical radiation therapy and chemotherapy for primary squamous cell carcinoma, 489
bone
chemotherapy for osteogenic sarcoma: an investigative method, not a recipe, 1687
ethics of not conducting a prospectively controlled trial of adjuvant chemotherapy in osteosarcoma, 1699
primary non-Hodgkin's lymphoma of: results in 15 patients treated by radiotherapy combined with systemic chemotherapy, 1859
and soft tissue sarcomas, gallium nitrate in advanced: a Southwest Oncology Group study, 1673
brain
carmustine (BCNU) and dianhydrogalactitol combination in the treatment of primary, recurring after irradiation, 1647
cerebrospinal fluid versus serum concentrations of 5-FU, allopurinol, and oxypurinol during treatment of metastatic, with 5-FU infusion, allopurinol, and radiation, 571
cisplatin therapy in recurrent childhood tumors, 2013
platinum concentrations in human glioblastoma multiforme following the use of cisplatin, 1661
proper analysis of clinical trials for malignant glioma, 211
breast
AMSA in patients with metastatic: a Cancer and Leukemia Group B phase II study, 1211
AMSA, prednisone, and chlorambucil (APC) in the treatment of refractory: a Northern California Oncology Group study, 1235
AMSA in refractory: phase II study, 403
anticipatory vomiting in women receiving cyclophosphamide, methotrexate, and 5-FU adjuvant chemotherapy for carcinoma, 1601
aziridinylbenzoquinone (AZQ) in advanced: a Cancer and Leukemia Group B phase II trial, 1875
chlorozotocin in malignant melanoma, other solid tumors, and: a phase I trial, 1229
3-deazaguanine: a candidate drug for the chemotherapy of carcinomas? 1853
doxorubicin as a single agent in patients with previously untreated: experience of the Eastern Cooperative Oncology Group, 1594
hydroxy-9-methyl-2-ellipticinium for osseous metastases from: a 5-year experience, 1909
Estracyt in advanced, a pilot study, 581
failure of 5-day vinblastine infusion in the treatment of patients with advanced refractory, 1783
gallium nitrate in: a Southwest Oncology Group phase II study, 1591
high-dose medroxyprogesterone acetate in advanced male, 1763
spirogermanium in adenocarcinoma: a Cancer and Leukemia Group B phase II study, 1667
tamoxifen before and after orchiectomy in advanced male, 1882
third-line chemotherapy with mitomycin, vinblastine, and carmustine (BCNU) in refractory carcinoma: a pilot study, 559
vincristine, doxorubicin, prednisolone, and cyclophosphamide (VAP-cyclo)—a short intensive regimen for advanced, 1999
vindesine in patients with advanced: a phase II study, 1729
bronchogenic
bleomycin, cisplatin, vincristine, and methotrexate in advanced non-small cell carcinoma, 205
combination chemotherapy for, with doxorubicin, BCNU, and cyclophosphamide (ABC): a pilot study of the Southeastern Cancer Study Group, 199
divided-dose vinblastine in carcinoma: a phase II study, 171

doxorubicin and mitomycin in non-small cell: a phase II study, 177
upper hemibody and local chest irradiation as consolidation following response to high-dose induction chemotherapy for small cell carcinoma—a pilot study, 1609
bronchial
combination chemotherapy with vincristine, carmustine (BCNU), and bleomycin in patients with squamous cell carcinoma resistant to cyclophosphamide, doxorubicin, methotrexate, and procarbazine (CAMP), 1877
tumor size, response rate, and prognosis in small cell carcinoma treated by combination chemotherapy, 463
carcinoma
multiple squamous cell, treated with intra-arterial cisplatin in a patient with rheumatoid arthritis, 1987
cervical
dianhydrogalactitol and cisplatin in combination for advanced, 1809
sequential combination of bleomycin and mitomycin in advanced: a Northern California Oncology Group study, 163
cholangiocarcinoma
response to doxorubicin and mitomycin: a case report, 209
colon
5-FU and allopurinol: toxicity modulation and phase II results in, 1133
methyl-GAG in advanced: a phase II trial of the Southwest Oncology Group, 2099
colorectal
bisantrene in advanced: a Cancer and Leukemia Group B phase II study, 1997
combination chemotherapy in patients with advanced, previously treated with 5-FU, 2093
4′-epi-doxorubicin in patients with advanced carcinoma: a phase II evaluation, 1757
and gastric adenocarcinomas, advanced, treatment with 5-FU combined with high-dose folinic acid (leucovorin): a pilot study, 1803
high-dose intermittent cycloleucine in: a phase II study, 203
mitoxantrone in advanced: a phase II study, 1995
mitoxantrone in patients with previously untreated adenocarcinoma: a Southwest Oncology Group phase II trial, 1779
sequential methotrexate-5-FU therapy in advanced measurable, 1563
sequential methotrexate and 5-FU in the treatment of, 1553
vindesine for patients with advanced carcinoma: a phase II evaluation, 583
endometrial
etoposide in the management of advanced or recurrent carcinoma: a Gynecologic Oncology Group phase II trial, 1669
esophageal
methyl-GAG in the treatment of carcinoma: a phase II study, 1427
gastrointestinal
advanced colorectal and gastric adenocarcinomas, treatment with 5-FU combined with high-dose folinic acid (leucovorin): a pilot study, 1803
anguidine in: a Southwest Oncology Group phase II study, 381
5-FU, doxorubicin, and mitomycin (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas: phase I–II study, 1613
randomized comparison of 5-FU alone or combined with mitomycin and cytarabine (MFC) in the treatment of advanced gastric, 1263
studies of Baker's antifol, methotrexate, and razoxane in advanced gastric cancer: a Gastrointestinal Tumor Study Group report, 1713
germ cell
VAB-3 combination chemotherapy in mediastinal tumors, 1405
head and neck
aziridinylbenzoquinone (AZQ) in: phase II trial, 2097
bleomycin and hydroxyurea combination chemotherapy in the treatment of, 1439
clinical trial of cisplatin and 5-FU infusion as initial treatment for advanced squamous cell carcinoma, 471
combination chemotherapy used prior to radiation therapy for locally advanced squamous cell carcinoma, 1421
methotrexate and 5-FU for recurrent, 1925
razoxane (ICRF-159) in patients with squamous cell carcinoma, previously exposed to systemic chemotherapy, 557
thioproline in advanced epidermoid: a phase II trial, 585
Hodgkin's disease *see* Hodgkin's disease
hypernephroma
bisantrene for advanced: a phase II trial, 1653
Kaposi's sarcoma
the epidemic of acquired immune dysfunction in homosexual men and its sequelae—opportunistic infections, Kaposi's sarcoma, and other malignancies: an update and interpretation, 1391
workshop on: meeting report, 1387
liver
m-AMSA in hepatocellular carcinoma: a Southwest Oncology Group phase II trial, 1651
isotopic immunoglobulin therapy for primary: a phase I–II study, 289
lung
CCNU, vincristine, methotrexate, and procarbazine treatment of relapsed small cell carcinoma, 1557
chemotherapy versus chemoimmunotherapy with levamisole or *Corynebacterium parvum* in advanced, 1291
cisplatin combination chemotherapy in non-oat cell carcinoma, 197
cisplatin, doxorubicin, cyclophosphamide, and vindesine combination chemotherapy for non-small cell, 247
cisplatin, maytansine, and chlorozotocin in small cell carcinoma: a phase II study, 1417
combination chemotherapy for small cell carcinoma: continuous versus alternating non-cross-resistant combinations, 221
deoxynorleucine (DON) in patients with previously treated advanced: a phase II study, 1665
extensive adenocarcinoma and large cell undifferentiated carcinoma treated with 5-FU, vindesine, and mitomycin (FEMi): a Southwest Oncology Group study, 553
high-dose cisplatin for, 375

intensive timed sequential combination chemotherapy in extensive-stage small cell carcinoma, 1880
lack of efficacy of high-dose methotrexate by 30-hour infusion in patients with progressive small cell carcinoma, 1223
leukemia occurring after treatment of small cell, 1683
limited squamous cell: a Southwest Oncology Group randomized study of radiation with or without doxorubicin chemotherapy and with or without levamisole immunotherapy, 1113
mitomycin, hexamethylmelamine, and vindesine as second-line treatment for small cell, 1873
non-cross-resistant chemotherapy and consolidation radiotherapy for small cell carcinoma, 1399
procarbazine in small cell carcinoma: a phase II study, 273
random prospective study of cyclophosphamide, doxorubicin, and methotrexate (CAM) combination chemotherapy versus single-agent sequential chemotherapy in non-small cell, 2005
randomized phase II comparison of methotrexate with or without L-asparaginase in non-small cell, 541
vindesine: an effective agent in the treatment of non-small cell, 1709
vindesine monochemotherapy for non-small cell: a report of 45 cases, 1409
vindesine in the treatment of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of, 305
lymphoepithelioma
combined chemotherapy with doxorubicin, bleomycin, vinblastine, and dacarbazine, and radiotherapy for advanced, 1207
lymphoma
AMSA therapy for children with lymphoblastic malignancy, 593
combination chemotherapy for patients with relapsed malignant, using methyl-GAG and teniposide (VM-26), 1121
comparative trial of two teniposide-based combination chemotherapy regimens for the treatment of advanced malignant, 2021
comparing doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine (ACOMLA) with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin (CHOP-B) in the treatment of diffuse, 1279
comparison of the use of teniposide and vincristine in combination chemotherapy for non-Hodgkin's, 43
cyclophosphamide and prednisolone high-dose regimen in advanced non-Hodgkin's, of favorable histologic type: phase II study, 377
deferral of initial therapy for advanced indolent, 417
maytansine in refractory non-Hodgkin's: a Southwest Oncology Group phase II study, 1587
methotrexate (COP or CHOP) combinations in the treatment of previously untreated and treated, 43
prognostic significance of the Lukes and Collins classification in patients treated with COMLA (cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine), 1107
role of combined modality therapy in the curative management of advanced-stage "favorable" non-Hodgkin's, 421
salvage chemotherapy for non-Hodgkin's, 1977
melanoma
AMSA in patients with stage III–IV malignant, 1237
an aziridinylbenzoquinone (AZQ) in disseminated malignant: a phase II study, 2089
chlorozotocin in breast cancer, other solid tumors, and malignant: phase I trial, 1229
chlorozotocin in malignant: a Southwest Cancer Study Group phase II study, 1431
cisplatin hydration with and without mannitol diuresis in refractory disseminated malignant: a Southwest Oncology Group study, 31
clinical trial of chlorozotocin, DTIC, and dactinomycin in malignant, 371
combination of vinblastine, bleomycin, and cisplatin in advanced malignant: a phase II study, 567
high-dose megestrol acetate in patients with advanced malignant: a phase II study, 1239
mitolactol in patients with metastatic: a phase II trial, 195
mitolactol and semustine combination chemotherapy for advanced malignant, 1425
serial analysis of growth kinetics in a patient receiving bleomycin and procarbazine therapy: comparison of tumor volume, flow cytometry, and thymidine labeling techniques, 529
tamoxifen in malignant: a phase II study, 1447
tamoxifen in metastatic, Southwest Oncology Group experience with, 1680
tamoxifen in patients with metastatic malignant: a phase II study, 1437
tamoxifen in the treatment of metastatic malignant, 1771
therapeutic effect of dopamine infusion on human malignant, 1657
treatment of advanced malignant, with vinblastine, bleomycin, and cisplatin, 1299
mesothelioma
doxorubicin and radiation therapy combination in malignant pleural, 1605
mycosis fungoides
combination chemotherapy with cyclophosphamide, vincristine, and prednisone (CVP) in TNM-classified stage IV, 167
myeloma
combination chemotherapy for multiple, with melphalan, prednisone, cyclophosphamide, vincristine, and BCNU (M-2 protocol), 1971
cisplatin, BCNU, cyclophosphamide, and prednisone in multiple, 237
resistant to melphalan: treatment with doxorubicin, cyclophosphamide, carmustine (BCNU), and prednisone, 451
treatment of advanced plasma cell, with weekly cyclophosphamide and alternate-day prednisone, 1413
vincristine, BCNU, doxorubicin, and prednisone (VBAP) combination in the treatment of relapsing or resistant multiple: a Southwest Oncology Group study, 1267
ovarian
AMSA in patients with advanced: a phase II study, 1242
AMSA in previously treated patients with stage III and IV: a phase II study, 1589
hexamethylmelamine-CAF (cyclophosphamide, metho-

trexate, and 5-FU) and cisplatin-CAF in refractory, 1233
hexamethylmelamine and cisplatin in, after failure of alkylating-agent therapy, 1285
hexamethylmelamine distribution in patients with, and other pelvic cancers, 231
high-dose medroxyprogesterone acetate (MPA) in advanced chemotherapy-resistant carcinoma: a phase II study, 407
high-dose medroxyprogesterone acetate for the treatment of advanced carcinoma, 1441
ip immunotherapy and chemotherapy in advanced epithelial, 1977
pharmacology and toxicity of ip streptozocin in: a case study, 1595
spirogermanium in advanced epithelial carcinoma: a phase II study, 1675
Yoshi 864 in epithelial carcinoma: a Gynecologic Oncology Group phase II clinical study, 1775
pancreatic
combination chemotherapy with 5-FU and 1-(aminomethyl)-3-(2-chloroethyl)-3-nitrosourea for carcinoma, 1645
doxorubicin therapy for advanced islet cell carcinoma: a phase II study, 1567
5-FU, doxorubicin, and mitomycin (FAM) and chlorozotocin in advanced measurable: a phase II study, 2095
radiation therapy combined with intra-arterial 5-FU chemotherapy for treatment of localized adenocarcinoma: a Southwest Oncology Group study, 549
prostatic
AMSA in: a phase II study, 208
m-AMSA in disseminated carcinoma: a phase II study, 1993
cisplatin, cyclophosphamide, and prednisone therapy for stage D, 1403
cyclophosphamide, doxorubicin, and methotrexate in the treatment of advanced, 1797
doxorubicin, mitomycin, and 5-FU in the treatment of hormone-resistant stage D: a preliminary report, 57
renal cell
bisantrene in patients with carcinoma: a phase II evaluation, 1869
dianhydrogalactitol in advanced carcinoma: a Southwest Oncology Group phase II study, 1231
medroxyprogesterone acetate with tamoxifen in advanced, 1773
soft tissue
and bone sarcomas, gallium nitrate in advanced: a Southwest Oncology Group study, 1673
evaluation of azapicyl in children with sarcoma: a report from the Children's Cancer Study Group, 399
prednimustine in advanced sarcoma, 201
T-cell
cytarabine in chronic neoplasms, 591
effect of cytarabine on development of cytotoxic, 535
testicular
clinical stage I: the medical oncologist's view, 15
introduction to current controversies in management of, 1
optimal management of clinical stage I nonseminomatous carcinoma: one oncologist's view, 11
persistent hypomagnesemia following cisplatin chemotherapy for, 1767
surgical treatment of clinical stage I nonseminomatous germ cell tumors, 5
urinary tract
4′-epi-doxorubicin in metastatic renal: a phase II study, 1219
management of pelvic complications of malignant urothelial tumors with combined intra-arterial and iv chemotherapy, 1501
methyl-GAG and vinblastine combination in the treatment of metastatic renal adenocarcinoma, 1585
PALA in hypernephroma and urinary bladder cancer: a phase II trial, 2091
VM-26 in patients with metastatic transitional cell carcinoma: an Eastern Cooperative Oncology Group phase II evaluation, 405
Yoshi 864 plus medroxyprogesterone in adenocarcinoma of the kidney: a Southwest Oncology Group study, 1781
Wilms' tumor
invited comment on the Milwaukee pilot study of doxorubicin-vincristine therapy for, 1795
vincristine-doxorubicin therapy for: a pilot study, 1791
Carmustine (BCNU)
with cisplatin, cyclophosphamide, and prednisone in multiple myeloma, 237
with cyclophosphamide and doxorubicin, combination chemotherapy for bronchogenic carcinoma: a pilot study of the Southeastern Cancer Study Group, 199
and dianhydrogalactitol combination in the treatment of primary brain tumors recurring after irradiation, 1647
with doxorubicin, cyclophosphamide, and prednisone in the treatment of multiple myeloma resistant to melphalan, 451
intermittent with cyclophosphamide and prednisone versus intermittent melphalan and prednisone in myeloma, 1273
with melphalan, prednisone, cyclophosphamide, and vincristine (M-2 protocol): combination chemotherapy for multiple myeloma, 1971
with mitomycin and vinblastine as third-line chemotherapy in refractory breast carcinoma: a pilot study, 559
with vincristine and bleomycin in patients with squamous cell bronchial carcinoma resistant to cyclophosphamide, doxorubicin, methotrexate, and procarbazine (CAMP), 1877
vincristine, doxorubicin, and prednisone (VBAP) combination in the treatment of relapsing or resistant multiple myeloma: a Southwest Oncology Group study, 1267
CCNU *see* lomustine
Cell culture studies
action of lycurim and pyrazofurin on hepatoma 3924A cells in culture, 343
chemotherapy sensitivity assessment of leukemic colony-forming cells with in vitro simultaneous exposure to multiple drugs: clinical correlations in acute nonlymphocytic leukemia, 1257
comparison of the effects on cultured L1210 leukemia cells of the ribosyl, 2′-deoxyribosyl, and xylosyl homologs and tubercidin and adenosine alone or in combination with 2′-deoxycoformycin, 317
comparison of thymidine and folinic acid protection from methotrexate toxicity in human lymphoid cell lines, 2051
development and characterization of a cyclophosphamide-

resistant mouse plasmacytoma cell line, 1535
doxorubicin cytotoxicity enhanced by local anesthetics in a human melanoma cell line, 121
increased relative effectiveness of doxorubicin against slowly proliferating Sarcoma 180 cells after prolonged drug exposure, 1159
modulation of cytarabine metabolism in the human promyelocytic leukemia cell line HL-60 by polyhydroxy-substituted benzohydroxamic acids, 1825
Cervical cancer *see* Cancer, cervical
Chemoimmunotherapy
for autochthonous acute rat leukemias, 391
Chemotherapy
changes in intestinal permeability to lactulose induced by cytotoxic, 1435
versus chemoimmunotherapy with levamisole or *Corynebacterium parvum* in advanced lung cancer, 1291
combination used prior to radiation therapy for locally advanced squamous cell carcinoma of the head and neck, 1421
intensive timed sequential, in extensive-stage small cell carcinoma of the lung, 1880
management of pelvic complications of malignant urothelial tumors with combined intra-arterial and iv, 1501
Chlorambucil
with AMSA and prednisone (APC) in the treatment of refractory breast cancer: a Northern California Oncology Group study, 1235
Chlorozotocin
with cisplatin and maytansine in small cell lung carcinoma (EST 2578): a phase II study, 1417
with DTIC and dactinomycin, clinical trial in metastatic malignant melanoma, 371
in malignant melanoma, breast cancer, and other solid tumors: phase I trial, 1229
in malignant melanoma: a Southeastern Cancer Study Group phase II study, 1431
Cholangiocarcinoma *see* Cancer, cholangiocarcinoma
Cisplatin
acute vascular episodes associated with therapy for carcinomas of the upper aerodigestive tract with bleomycin, vincristine, and, 369
antiemetic activity of butorphanol against emesis induced by, in ferrets and dogs, 1831
antitumor activity and toxicity of analogs of, 135
with BCNU, cyclophosphamide, and prednisone in multiple myeloma, 237
with bleomycin, vincristine, and methotrexate in advanced non-small cell bronchogenic carcinoma, 205
clinical trial of 5-FU infusion and, as initial treatment for advanced squamous cell carcinoma of the head and neck, 471
combination chemotherapy in non-oat cell carcinoma of the lung, 197
combination with vinblastine and bleomycin in advanced malignant melanoma: a phase II study, 567
with cyclophosphamide, methotrexate, and 5-FU and hexamethylmelamine-CAF in refractory ovarian cancer, 1233
with cyclophosphamide and prednisone, therapy for stage D prostatic cancer, 1403
and dianhydrogalactitol in combination for advanced cancer of the uterine cervix, 1809
with doxorubicin, cyclophosphamide, and vindesine, combination chemotherapy for non-small cell lung cancer, 247
epileptic seizures associated with administration of, 1719
experimental ultrastructural and X-ray microanalysis study of, in the rat: intracellular localization of platinum, 1529
and hexamethylmelamine in advanced ovarian cancer after failure of alkylating-agent therapy, 1285
high-dose for lung cancer, 375
hydration with and without mannitol diuresis in refractory malignant melanoma: a Southwest Oncology Group study, 31
-induced emesis in the ferret: a new animal model, 187
-induced emesis, results of a randomized double-blind crossover trial of scopolamine versus placebo administered by transdermal patch for the control of, 1975
-induced nausea, effective control using high-dose steroids and droperidol, 243
with maytansine and chlorozotocin in small cell lung carcinoma (EST 2578): a phase II study, 1417
multiple squamous cell carcinoma treated with intra-arterial, in a patient with rheumatoid arthritis, 1987
ototoxicity in patients receiving: importance of dose and method of drug administration, 19
persistent hypomagnesemia following chemotherapy with, for testicular cancer, 1767
platinum concentrations in human glioblastoma multiforme following the use of, 1661
in recurrent childhood brain tumors, 2013
urinary pharmacokinetics and nephrotoxicity: a common circadian mechanism, 1933
with vinblastine and bleomycin, treatment of advanced malignant melanoma with, 1299
Clinical trials
chemotherapy for osteogenic sarcoma: an investigative method, not a recipe, 1687
a data-based approach to assessing clinical interventions in the setting of chronic disease, 1077
design of controlled clinical trials: use of historical controls, 1089
editorial on management of drug toxicity, 1901
ethics of not conducting a prospectively controlled trial of adjuvant chemotherapy in osteosarcoma, 1699
historical controls, data banks, and randomized trials in clinical research: a review, 1101
nonparametric confidence limits for survival probabilities and median survival time, 37
oral complications of cancer chemotherapy: present status and future studies, 1251
proper analysis of clinical trials for malignant glioma, 211
randomized clinical trials and research strategy, 1083
rationale for the use of alternative non-cross-resistant chemotherapy, 439
statistical guidelines for medical research reports, 217
strategy and alternate randomized designs in cancer clinical trials, 1095
Colon cancer *see* Cancer, colon
Colorectal cancer *see* Cancer, colorectal
COMLA (cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine)
prognostic significance of the Lukes and Collins classification in patients treated with, 1107

Corynebacterium parvum
or levamisole chemoimmunotherapy versus chemotherapy in advanced lung cancer, 1291
Cycloleucine
intermittent high-dose in colorectal malignancies: a phase II study, 203
Cyclophosphamide
anticipatory vomiting in women with breast carcinoma receiving adjuvant chemotherapy with methotrexate, 5-FU, and, 1601
apparent sensitivity to: urticaria associated with cyclophosphamide without prior sensitization, 2106
with BCNU and doxorubicin, combination chemotherapy for bronchogenic carcinoma: a pilot study of the Southeastern Cancer Study Group, 199
with cisplatin, BCNU, and prednisone in multiple myeloma, 237
with cisplatin, doxorubicin, and vindesine, combination chemotherapy for non-small cell lung cancer, 247
with cisplatin and prednisone, therapy for stage D prostatic cancer, 1403
the course of nausea and vomiting after high-dose, 1487
with doxorubicin, carmustine (BCNU), and prednisone in the treatment of multiple myeloma resistant to melphalan, 451
with doxorubicin and methotrexate (CAM), random prospective study of combination chemotherapy versus single-agent sequential chemotherapy in non-small cell lung cancer, 2005
with doxorubicin and methotrexate in the treatment of advanced prostate cancer, 1797
with doxorubicin, vincristine, methotrexate with leucovorin rescue, and cytarabine, compared with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin in the treatment of diffuse histiocytic lymphoma, 1279
and ftorafur combination chemotherapy against advanced L1210 murine leukemia, 1631
with hexamethylmelamine, methotrexate, and 5-FU and cisplatin-CAF in refractory ovarian cancer, 1233
intermittent with carmustine (BCNU) and prednisone versus intermittent melphalan and prednisone in myeloma, 1273
-induced bladder toxicity, influence of diuretics on urinary general base catalytic activity and, 1889
with melphalan, prednisone, vincristine, and BCNU (M-2 protocol): combination chemotherapy for multiple myeloma, 1971
and prednisolone, high-dose regimen in advanced non-Hodgkin's lymphoma of favorable histologic type, 377
-resistant cell line, development and characterization of, 1535
transplacental passage of, 1681
with vincristine, doxorubicin, and prednisolone (VAP-Cyclo): a short intensive regimen for advanced breast cancer, 1999
with vincristine and prednisone (CVP), combination chemotherapy in TNM-classified stage IV mycosis fungoides, 167
vomiting induced by phosphoramide mustard and, in cats, 1625
weekly treatment of advanced plasma cell myeloma with, and alternate-day prednisone, 1413
Cytarabine
in chronic T-cell neoplasms, 591
with doxorubicin, cyclophosphamide, vincristine, and methotrexate with leucovorin rescue, compared with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin in the treatment of diffuse histiocytic lymphoma, 1279
effects compared with two nonclassic folic acid antagonists and methotrexate on hematopoietic precursor cells, 499
effect on cytotoxic T-cell development, 535
metabolism modulation in the human promyelocytic leukemia cell line HL-60 by polyhydroxy-substituted benzohydroxamic acids, 1825
with mitomycin and 5-FU, randomized comparison in the treatment of advanced gastric cancer, 1263
and pyrazofurin, treatment of refractory acute nonlymphocytic leukemia with a combination of, 1397
response of hypereosinophilic syndrome to 6-thioguanine and, 545
Cytosine arabinoside *see* Cytarabine
Cytoxan *see* Cyclophosphamide

D

Dacarbazine (DTIC)
with chlorozotocin and dactinomycin, clinical trial in metastatic malignant melanoma, 371
combined chemotherapy with doxorubicin, bleomycin, and vinblastine, and radiotherapy for advanced lymphoepithelioma, 1207
control of emesis induced by, with high-dose metoclopramide in a preliminary trial, 2108
Dactinomycin
with chlorozotocin and DTIC, clinical trial in metastatic malignant melanoma, 371
DAG *see* Dianhydrogalactitol
Daunomycin *see* Daunorubicin
Daunorubicin
cellular and plasma kinetics of, given by two methods of administration in a patient with acute leukemia, 2085
comparative cardiac and renal toxicity of, in the rat and rabbit, 395
toxicity reduction by conjugation to dextran, 107
10-Deaza-aminopterin
biochemical and pharmacologic properties of a new folate analog, 351
3-Deazaguanine
a candidate drug for the chemotherapy of breast carcinomas? 1853
3-Deazauridine
daily × 5 schedule of: phase I trial and pharmacokinetics of, 81
Decadron *see* Dexamethasone
2′-Deoxycoformycin
and 9-β-D-arabinofuranosyladenine (Ara-A) in the treatment of refractory acute myelocytic leukemia, 253
comparison of the effects on cultured L1210 leukemia cells of the ribosyl, 2′-deoxyribosyl, and xylosyl homologs and tubercidin and adenosine alone or in combination with, 317

relationship of 5′-nucleotidase activity and antileukemic effect in therapy with, 387
5′-Deoxy-5-fluorouridine
a new fluoropyrimidine derivative: a phase I clinical study, 1307
Depo Provera *see* Medroxyprogesterone
DES *see* Diethylstilbestrol
Dextran
toxicity reduction of daunorubicin by conjugation to, 107
Dianhydrodulcitol *see* Dianhydrogalactitol
Dianhydrogalactitol (DAG)
in advanced renal cell carcinoma: a Southwest Oncology Group phase II trial, 1231
and carmustine (BCNU) combination in the treatment of primary brain tumors recurring after irradiation, 1647
and cisplatin in combination for advanced cancer of the uterine cervix, 1809
clinical and pharmacologic evaluation of split-dose intermittent therapy with, 283
Dibromodulcitol *see* Mitolactol
Dihydrofolate reductase
malnutrition effect on tissue levels of, and toxicity of methotrexate, 85
Dihydroxyanthracenedione (DHAD) *see* Mitoxantrone
Dimethyl sulfoxide (DMSO)
prevention of doxorubicin-induced skin ulcers in the rat and pig with, 1371
DNA
-doxorubicin complex: a phase I clinical trial, 2033
DON (6-diazo-5-*oxo*-norleucine)
administered by 5-day courses: a phase I trial, 1215
in patients with previously treated advanced lung cancer: a phase II study, 1665
Dopamine
therapeutic effect of infusion on human malignant melanoma, 1657
Doxorubicin
altered hepatic disposition of, in the perfused rat liver at hyperthermic temperatures, 1357
with BCNU and cyclophosphamide, combination chemotherapy for bronchogenic carcinoma: a pilot study of the Southeastern Cancer Study Group, 199
with cisplatin, cyclophosphamide, and vindesine, combination chemotherapy for non-small cell lung cancer, 247
combined chemotherapy with bleomycin, vinblastine, and dacarbazine, and radiotherapy for advanced lymphoepithelioma, 1207
with cyclophosphamide, carmustine (BCNU), and prednisone in the treatment of multiple myeloma resistant to melphalan, 451
with cyclophosphamide and methotrexate (CAM), random prospective study of combination chemotherapy versus single-agent sequential chemotherapy in non-small cell lung cancer, 2005
with cyclophosphamide and methotrexate in the treatment of advanced prostate cancer, 1797
with cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine, compared with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin in the treatment of diffuse histiocytic lymphoma, 1279
cytotoxicity enhanced by local anesthetics in a human melanoma cell line, 121
-DNA complex: a phase I clinical trial, 2033
4′-epi- derivative of, activity on normal hematopoietic precursor cells in mice, 2061
4′-epi- derivative in metastatic renal cancer: a phase II study, 1219
4′-epi- derivative in patients with advanced colorectal carcinoma: a phase II study, 1757
4′-epi- derivative, pharmacokinetic studies in cancer patients with normal and impaired renal function and with hepatic metastases, 1819
with 5-FU and mitomycin (FAM) and chlorozotocin, a phase II study in advanced measurable pancreatic cancer, 2095
with 5-FU, mitomycin, and semustine (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas: a phase I–II study, 1613
ganglioneuropathy in rabbits and rhesus monkey due to high cumulative doses of, 1349
high-dose: an exploration of the dose-response curve in human neoplasia, 493
increased relative effectiveness of, against slowly proliferating Sarcoma 180 cells after prolonged drug exposure, 1159
-induced skin ulcers in the rat and pig, prevention with dimethyl sulfoxide (DMSO), 1371
inhibition of cardiac guanylate cyclase by, and some of its analogs: possible relationship to cardiotoxicity, 311
local and systemic toxicity resulting from large-volume ip administration in the rat, 157
with mitomycin and 5-FU in the treatment of hormone-resistant stage D prostate cancer: a preliminary report, 57
and mitomycin in non-small cell bronchogenic carcinoma: a phase II study, 177
and mitomycin, response in cholangiocarcinoma: a case report, 209
with or without radiation and with or without levamisole immunotherapy in limited squamous cell carcinoma of the lung: a Southwest Oncology Group randomized study, 1113
with radiation therapy in malignant pleural mesothelioma, 1605
safety compared to mitoxantrone, a new anticancer compound: clinical observations, 1139
safety compared to mitoxantrone, a new anticancer compound: histologic and ultrastructural pathology, 1145
as a single agent in patients with previously untreated breast cancer: experience of the Eastern Cooperative Oncology Group, 1594
subcutaneous reaction due to intra-arterial infusion of, 2000
therapy for advanced islet cell carcinoma: a phase II study, 1567
vincristine, BCNU, and prednisone (VBAP) combination in the treatment of relapsing or resistant multiple myeloma: a Southwest Oncology Group study, 1267
with vincristine, prednisolone, and cyclophosphamide (VAP-cyclo): a short intensive regimen for advanced breast cancer, 1999
-vincristine therapy for Wilms' tumor: invited comment on the Milwaukee pilot study, 1795
-vincristine therapy for Wilms' tumor: a pilot study, 1791

Droperidol
effective control of cisplatin-induced nausea using high-dose steroids and, 243
DTIC *see* Dacarbazine

E

Ellipticine
isolation and properties of Chinese hamster lung cells resistant to derivatives of, 327
Ellipticinium
hydroxy-9-methyl-2- derivative for osseous metastases from breast cancer: a 5-year experience, 1909
Endoxan *see* Cyclophosphamide
Esophageal cancer *see* Cancer, esophageal
Estracyt
in advanced breast cancer, a pilot study, 581
Etoposide (VP-16-213)
in the management of advanced or recurrent endometrial carcinoma: a Gynecologic Oncology Group phase II study, 1669
treatment of the blast crisis of chronic myelogenous leukemia with 5-azacitidine and, 267
unusual reaction to, and avoidance by prolonged infusion, 409
Etoprine
and metoprine, evaluation in the avian sarcoma virus glioma, rat model, 99

F

FAM (5-FU, doxorubicin, and mitomycin)
and chlorczotocin, a phase II study in advanced measurable pancreatic cancer, 2095
5-Fluorouracil *see* 5-FU
5-FU
with 1-(aminomethyl)-3-(2-chloroethyl)-3-nitrosourea (ACNU) and mitomycin (FUM) for carcinoma of the pancreas, 1645
and allopurinol: toxicity modulation and phase II results in colon cancer, 1133
alone or combined with mitomycin and cytarabine (MFC), randomized comparison in the treatment of advanced gastric cancer, 1263
anticipatory vomiting in women with breast carcinoma receiving adjuvant chemotherapy with cyclophosphamide, methotrexate and, 1601
cerebrospinal fluid versus serum concentrations of allopurinol, oxypurinol, and, during treatment of metastatic brain cancer with 5-FU infusion, allopurinol, and radiation, 571
clinical trial of cisplatin and, as initial treatment for advanced squamous cell carcinoma of the head and neck, 471
combined with hypoxanthine and allopurinol in chemotherapy for human colon adenocarcinoma xenografts, 1201
combination chemotherapy in patients with advanced colorectal carcinoma previously treated with, 2093
combination therapy with continuous-infusion PALA and bolus-injection: a phase II trial, 200
with doxorubicin and mitomycin (FAM) and chlorozotocin, a phase II study in advanced measurable pancreatic cancer, 2095
with doxorubicin, mitomycin, and semustine (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas: a phase I–II study, 1613
effects on hematopoietic stem cells in Friend murine leukemia virus-infected mice undergoing chemotherapy, 1949
with hexamethylmelamine, cyclophosphamide, methotrexate, and cisplatin-CAF in refractory ovarian cancer, 1233
with high-dose folinic acid (leucovorin) for treatment of advanced colorectal and gastric adenocarcinomas: a pilot study, 1803
intra-arterial chemotherapy combined with radiation therapy for treatment of localized adenocarcinoma of the pancreas: a Southwest Oncology Group study, 549
and methotrexate for recurrent head and neck cancer, 1925
-methotrexate sequential therapy in advanced measurable colorectal cancer: a phase II study, 1563
and methotrexate, sequentially, in the treatment of colorectal cancer, 1553
with mitomycin and doxorubicin in the treatment of hormone-resistant stage D prostate cancer: a preliminary report, 57
modulation of toxicity of, with high-dose allopurinol: phase I trial of an outpatient dose schedule, 1723
nasal cartilage necrosis following high-dose: a case report, 1884
and PALA, pilot study in patients with advanced cancer, 77
with prednimustine and methotrexate (PMF): a pilot study, 2081
with vindesine and mitomycin (FEMi) in the treatment of extensive adenocarcinoma and large cell undifferentiated carcinoma of the lung: a Southwest Oncology Group study, 553
Folinic acid *see* Leucovorin
Ftorafur
and cyclophosphamide combination chemotherapy against advanced L1210 murine leukemia, 1631

G

Gallium nitrate
in advanced soft tissue and bone sarcomas: a Southwest Oncology Group study, 1673
in breast cancer: a Southwest Oncology Group phase II study, 1591
Gastrointestinal cancer *see* Cancer, gastrointestinal
Germ cell cancer *see* Cancer, germ cell
Glutaminase-asparaginase
succinylated *Acinetobacter*, clinical evaluation in adult leukemia, 1479
Glycine
N-diazoacetyl derivatives of phenylalanine and, antitumor activity against P388 leukemia and B16 melanoma in mice, 179
Guanylate cyclase
inhibition of cardiac, by doxorubicin and some of its analogs: possible relationship to cardiotoxicity, 311

lack of correlation between effects of anthracyclines on in vitro cardiac, and on in vivo cardiotoxicity, 1677

H

Head and neck cancer *see* Cancer, head and neck
Hexamethylmelamine
-CAF (cyclophosphamide, methotrexate, and 5-FU) and cisplatin-CAF in refractory ovarian cancer, 1233
and cisplatin in advanced ovarian cancer after failure of alkylating-agent therapy, 1285
distribution in patients with ovarian and other pelvic cancers, 231
with mitomycin and vindesine as second-line treatment for small cell cancer of the lung, 1873
parenteral formulation of, potentially suitable for use in man, 1579
and pentamethylmelamine, tissue distribution in M5076/73A ovarian cancer-bearing mice, 127
Hodgkin's disease
anatomic substages of stage IIIA: followup of a collaborative study, 733
characteristics of cell lines, 615
changing patterns of, at autopsy: a 25-year experience at the National Cancer Institute, 1953–1978, 653
chemotherapy for advanced: conclusions from the Southeastern Cancer Study Group, 899
chemotherapy and combined modality therapy for: a progress report on Cancer and Leukemia Group B studies, 835
chemotherapy for: the remaining challenges, 925
in children: treatment results with MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) and low-dose, extended-field irradiation, 949
chromosomes in, 639
collaborative clinical trial for stage I and II: significance of mediastinal and nonmediastinal disease in laparotomy- and nonlaparotomy-staged patients, 775
combined modality therapy for advanced: long-term followup data, 871
complications of treatment of, in children, 977
conclusions from clinical trials of the Southwest Oncology Group, 847
cyclic delivery of MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) and ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) combinations in stage IV: rationale, background studies, recent results, 881
with direct extension into pulmonary parenchyma from a mediastinal mass: a presentation requiring special therapeutic considerations, 717
distinguishing features of the immunology of, in children, 969
effect of age on therapeutic response and survival in advanced, 889
growth factors in, 633
histochemistry of, 609
imaging techniques for staging abdominal, 697
immunohistochemical analysis of the distribution of lymphocyte subpopulations in, 667
immune status of untreated patients with, and prognosis, 701
implications of persistent T cell abnormalities for the etiology of, 681
influence of mediastinal adenopathy on site and frequency of relapse in patients with, 809
intergroup study of stages I and II in children: a preliminary report, 937
late effects of radiation therapy in the treatment of, 991
long-term cardiovascular evaluation of patients with, treated by thoracic mantle radiation therapy, 1003
long-term complications of MOPP chemotherapy in patients with, 1015
mistaken clinical and pathologic diagnosis of: a Southwest Oncology Group Study, 645
multidisciplinary treatment of advanced, by an alternating chemotherapeutic regimen of MOPP/ABVD and low-dose radiation therapy restricted to originally bulky disease, 907
patterns of care outcome studies in: results of the national practice and implications for management, 805
pathologist's role in management of patients with, 675
posttreatment laparotomy as a guide to management in patients with, 759
possible origin of the Reed-Sternberg cell from an interdigitating reticulum cell, 601
prognostic factors in pathologic stage III, 743
prognostic significance of mediastinal mass in childhood, 961
radiotherapy versus combined modality treatment of stage I and II, 799
results of lung irradiation for patients with large mediastinal masses and/or hilar disease, 819
results of treating without a policy of laparotomy staging, 717
role of radiation therapy in the treatment of, 915
second malignancies complicating: a Southwest Oncology Group 10-year followup, 1023
second malignant neoplasms in patients successfully treated for: a Cancer and Leukemia Group B study, 1035
social class and age at diagnosis of: new epidemiologic evidence for the two disease hypothesis, 689
stage I and II: involved-field radiotherapy versus extended-field radiotherapy versus involved-field radiotherapy followed by six cycles of MOPP (mechlorethamine, vincristine, prednisone, and procarbazine), 789
strategic role of laparotomy in staging, 767
summary and future directions, 1045
surgical restaging of, 751
treatment of advanced: 10-year experience in the Eastern Cooperative Oncology Group, 855
treatment of stage IIIA, 827
Hydroxydaunorubicin *see* Doxorubicin
7-Hydroxymethotrexate
and methotrexate, pharmacokinetics following infusions of high-dose methotrexate, 1733
Hydroxyurea
and bleomycin combination chemotherapy in the treatment of advanced head and neck cancer, 1439
Hyperthermia
ultrasonic, and drugs in the therapy for human tumor xenografts, 1635

whole-body, and chemotherapy for treatment of patients with advanced, refractory malignancies, 259
Hypoxanthine
combined with 5-FU and allopurinol in chemotherapy for human colon adenocarcinoma xenografts, 1201

I

ICRF-159 *see* Razoxane
Imidazole-4-carboxamide dimethyltriazene *see* Dacarbazine
Immunotherapy
ip and chemotherapy in advanced epithelial ovarian cancer, 1981
radiation with or without doxorubicin chemotherapy and with or without levamisole, in limited squamous cell carcinoma of the lung: a Southwest Oncology Group randomized study, 1113
Staphylococcus aureus immunoperfusion: a phase I trial, 2027
Indicine-*N*-oxide
disposition in mice and monkeys, 183
Inosine dialdehyde *see* Diglycoaldehyde
Interferon
effects on C1300 mouse neuroblastoma, 1575
Isopropylamine platinum
studies on the pharmacokinetics and metabolism of, 509
Isotretinoin
activity against squamous cell cancers and preneoplastic lesions, 1315

K

Kaposi's sarcoma *see* Cancer, Kaposi's sarcoma

L

Lactulose
changes in intestinal permeability to, induced by cytotoxic chemotherapy, 1435
Leucovorin
comparison of protection from methotrexate toxicity by thymidine and, in human lymphoid cell lines, 2051
failure to reduce toxicity in massive vincristine overdose, 1967
high-dose, and 5-FU for treatment of advanced colorectal and gastric adenocarcinomas: a pilot study, 1803
rescue with methotrexate, doxorubicin, cyclophosphamide, vincristine, and cytarabine, compared with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin in the treatment of diffuse histiocytic lymphoma, 1279
Leukemia
acute
AMSA—a promising new agent in refractory, 1475
cellular and plasma kinetics of daunorubicin given by two methods of administration in a patient with, 2085
high-dose AMSA and autologous bone marrow transplantation in relapsed, 1991
trial of AMSA in 1596
adult
aclacinomycin A, a new anthracycline antibiotic: phase I–II evaluation in adults with refractory, 1619
succinylated *Acinetobacter* glutaminase-asparaginase in, clinical evaluation, 1479
central nervous system
aziridinylbenzoquinone (AZQ) treatment of, 2105
lymphoblastic
AMSA therapy for children with lymphoblastic malignancy, 593
nonlymphoblastic
results of aggressive chemotherapy for acute, in adults, 1917
marrow transplantation for patients with acute, who achieve a first remission, 1463
nonlymphocytic
5-azacitidine and zorubicin for patients with previously treated acute: a Cancer and Leukemia Group B pilot study, 563
chemotherapy sensitivity assessment of leukemic colony-forming cells with in vitro simultaneous exposure to multiple drugs: clinical correlations in acute, 1257
pyrazofurin and cytarabine combination in the treatment of refractory acute, 1397
myelocytic
comments on new approaches to therapy of acute, 1073
2′-deoxycoformycin (DCF) and 9-β-D-arabinosylfuranosyladenine (ara-A) in the treatment of refractory acute, 253
therapy for patients with acute, who enter remission: bone marrow transplantation or chemotherapy, 1467
myelogenous
role of intensive postinduction chemotherapy in the management of patients with acute, 1455
treatment of the blast crisis of chronic, with 5-azacitidine and VP-16-213, 267
myeloid
autologous bone marrow transplantation in acute, in relapse or in complete remission, 1983
Leukeran *see* Chlorambucil
Levamisole
or *Corynebacterium parvum* chemoimmunotherapy versus chemotherapy in advanced lung cancer, 1291
Liver cancer *see* Cancer, liver
Lomustine (CCNU)
inadvertent overdose with, followed by hematologic recovery, 1241
pulmonary fibrosis associated with: a case report, 1881
with vincristine, methotrexate, and procarbazine, treatment of relapsed small cell lung carcinoma, 1557
Lung cancer *see* Cancer, lung
Lycurim
and pyrazofurin, action on hepatoma 3924A cells in culture, 343
Lymphoepithelioma *see* Cancer, lymphoepithelioma
Lymphoma *see* Cancer, lymphoma

Malnutrition
effect on methotrexate toxicity and tissue levels of dihydrofolate reductase in the rat, 85
Mannitol
cisplatin hydration with and without diuresis in refractory malignant melanoma: a Southwest Oncology Group study, 31
Marihuana
contamination of cigarettes with pathogenic bacteria—possible source of infection in cancer patients, 589
Matulane *see* Procarbazine
Maytansine
with cisplatin and chlorozotocin in small cell lung carcinoma (EST 2578): a phase II study, 1417
in refractory non-Hodgkin's lymphoma: a Southwest Oncology Group phase II study, 1587
Medroxyprogesterone
acetate (MPA), high-dose, in advanced chemotherapy-resistant ovarian carcinoma: a phase II study, 407
acetate (MAP), relative bioavailability after single high-dose administration in cancer patients, 2043
high-dose, in advanced male breast cancer, 1763
high-dose, for the treatment of advanced ovarian carcinoma, 1441
with tamoxifen in advanced renal cell cancer: a phase II study, 1773
and Yoshi 864 in adenocarcinoma of the kidney: a Southwest Oncology Group study, 1781
Megestrol acetate
in patients with advanced malignant melanoma: a phase II study, 1239
Melanoma *see* Cancer, melanoma
Melphalan
enhanced lymph node uptake of, following liposomal entrapment and effects on lymph node metastases in rats, 517
essential thrombocythemia: response during first year of therapy with radioactive phosphorus and: a Polycythemia Vera Study Group report, 1495
influence of renal failure on myelosuppressive effects of: Cancer and Leukemia Group B experience, 475
intermittent with prednisone versus intermittent carmustine (BCNU), cyclophosphamide, and prednisone in myeloma, 1273
multiple myeloma resistant to: treatment with doxorubicin, cyclophosphamide, carmustine (BCNU), and prednisone, 451
with prednisone, cyclophosphamide, vincristine, and BCNU (M-2 protocol): combination chemotherapy for multiple myeloma, 1971
6-Mercaptopurine
fever due to, 1785
Methotrexate
anticipatory vomiting in women with breast carcinoma receiving adjuvant chemotherapy with cyclophosphamide, 5-FU and, 1601
with or without L-asparaginase: randomized phase II comparison in non-small cell cancer of the lung, 541
with bleomycin, cisplatin, and vincristine in advanced non-small cell bronchogenic carcinoma, 205
with CCNU, vincristine, and procarbazine, treatment of relapsed small cell lung carcinoma, 1557
combinations (COP or CHOP) in the treatment of previously untreated and treated lymphomas, 43
with cyclophosphamide and doxorubicin (CAM), random prospective study of combination chemotherapy versus single-agent sequential chemotherapy in non-small cell lung cancer, 2005
with cyclophosphamide and doxorubicin in the treatment of advanced prostate cancer, 1797
effects compared with two nonclassic folic acid antagonists and cytarabine on hematopoietic precursor cells, 499
effect of malnutrition on toxicity of, and tissue levels of dihydrofolate reductase in the rat, 85
and 5-FU for recurrent head and neck cancer, 1925
-5-FU sequential therapy in advanced measurable colorectal cancer: a phase II study, 1563
and 5-FU, sequentially, in the treatment of colorectal cancer, 1553
with hexamethylmelamine, cyclophosphamide, and 5-FU and cisplatin-CAF in refractory ovarian cancer, 1233
and 7-hydroxymethotrexate, pharmacokinetics following infusions of high-dose methotrexate, 1733
lack of efficacy of high-dose, by 30-hour infusion in patients with progressive small cell carcinoma of the lung, 1223
with leucovorin rescue, doxorubicin, cyclophosphamide, vincristine, and cytarabine, compared with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin in the treatment of diffuse histiocytic lymphoma, 1279
neurologic and cardiac toxicity following iv application of, 1561
with prednimustine and 5-FU (PMF): a pilot study, 2081
studies of Baker's antifol, razoxane and, in advanced gastric cancer: a Gastrointestinal Tumor Study Group report, 1713
treatment of neoplastic meningitis with intraventricular thiotepa and, 1549
Methyl-CCNU *see* Semustine
***N*-Methylformamide**
studies of the pharmacology of, in mice, 1957
Methyl-GAG
in advanced colon cancer: a phase II trial of the Southwest Oncology Group, 2099
combination chemotherapy for patients with relapsed malignant lymphoma using teniposide (VM-26) and, 1121
in patients with malignant neoplasms: a phase I re-evaluation, 65
in the treatment of esophageal carcinoma: a phase II study, 1427
and vinblastine combination in the treatment of metastatic renal adenocarcinoma, 1585
Metoclopramide
control of dacarbazine-induced emesis with high-dose, in a preliminary trial, 2108
high-dose, in cancer chemotherapy-induced nausea and vomiting, 2107
Metoprine
and etoprine, evaluation in the avian sarcoma virus glioma, rat model, 99
Misonidazole
-induced peripheral neuropathy, dose-response curve for predicting, 1743

O-demethylation of, by rat liver microsomes, 1343
Mitolactol
in patients with metastatic melanoma: a phase II trial, 195
and semustine combination chemotherapy for advanced malignant melanoma: a phase II study, 1425
Mitomycin
with bleomycin, sequential combination in advanced cervical cancer: a Northern California Oncology Group study, 163
with cytarabine and 5-FU, randomized comparison in the treatment of advanced gastric cancer, 1263
with doxorubicin and 5-FU in the treatment of hormone-resistant stage D prostate cancer: a preliminary report, 57
and doxorubicin in non-small cell bronchogenic carcinoma: a phase II study, 177
and doxorubicin, reponse in cholangiocarcinoma: a case report, 209
with 5-FU and 1-(aminomethyl)-3-(2-chloroethyl)-3-nitrosourea (ACNU) (FUM) for carcinoma of the pancreas, 1645
with 5-FU and doxorubicin (FAM) plus chlorozotocin, a phase II study in advanced measurable pancreatic cancer, 2095
with 5-FU, doxorubicin, and semustine (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas: phase I–II study, 1613
with 5-FU and vindesine (FEMi) in the treatment of extensive adenocarcinoma and large cell undifferentiated carcinoma of the lung: a Southwest Oncology Group study, 553
hemolytic-uremic syndrome associated with therapy, 457
with hexamethylmelamine and vindesine as second-line treatment of small cell cancer of the lung, 1873
-induced hemolytic uremic syndrome: case presentation and review of literature, 1244
with vinblastine and carmustine (BCNU) as third-line chemotherapy in refractory breast carcinoma: a pilot study, 559
Mitotane
modification of radiation damage in rat spinal cord by, 1545
Mitoxantrone
in advanced colorectal cancer: a phase II study, 1995
clinical and pharmacologic studies of, 1327
5-day schedule of: a phase I study, 1303
a phase II trial of, 1929
potential cardiotoxicity with, 1641
in previously untreated patients with colorectal adenocarcinoma: a Southwest Oncology Group phase II study, 1779
safety compared to doxorubicin: clinical observations, 1139
safety compared to doxorubicin: histologic and ultrastructural pathology, 1145
MOPP (mechlorethamine, vincristine, prednisone, and procarbazine)
multidisciplinary treatment of advanced Hodgkin's disease by alternating regimen of ABDV/MOPP and low-dose restricted radiation therapy, 907
stage I and II Hodgkin's disease: involved-field radiotherapy versus extended-field radiotherapy versus involved-field radiotherapy followed by six cycles of, 789
treatment results with low-dose, extended-field irradiation and, in childhood Hodgkin's disease, 949
Mycosis fungoides *see* Cancer, mycosis fungoides
Myeloma *see* Cancer, myeloma
Myleran *see* Busulfan

N

Neocarzinostatin *see* Zinostatin
Nitrogen mustard *see* Mechlorethamine
Nolvadex *see* Tamoxifen

O

Oncovin *see* Vincristine
Organ culture studies
prevention and reversal by a retinoid of 3,4-benzpyrene and cigarette smoke condensate-induced hyperplasia and metaplasia of rodent respiratory epithelia in, 1375
Ovarian cancer *see* Cancer, ovarian
Oxypurinol
cerebrospinal fluid versus serum concentrations of 5-FU, allopurinol and, during treatment of metastatic brain cancer with 5-FU infusion, allopurinol, and radiation, 571

P

PALA
and 5-FU: a phase II trial of combination therapy with bolus-injection 5-FU and continuous-infusion, 299
and 5-FU: pilot study in patients with advanced cancer, 77
in hypernephroma and urinary bladder cancer: a phase II trial, 2091
Pancreatic cancer *see* Cancer, pancreatic
Pentamethylmelamine
and hexamethylmelamine, tissue distribution in M5076/73A ovarian cancer-bearing mice, 127
a phase I trial of, 1227
Peplomycin
development and application of a radioimmunoassay for, 1365
Pharmacokinetics
of acivicin in plasma and cerebrospinal fluid, in Ommaya reservoir-bearing rhesus monkeys, 1333
of aziridinylbenzoquinone, clinical and pharmacologic studies, 1321
cisplatin nephrotoxicity and: a common circadian mechanism, 1933
dose-response curves for predicting misonidazole-induced peripheral neuropathy, 1743
doxorubicin 4′-epi derivative in cancer patients with normal and impaired renal function and with hepatic metastases, 1819
of high-dose methotrexate, effect of activated charcoal, 1169
and metabolism of *cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) in the dog, 509
methotrexate and 7-hydroxymethotrexate following infusions of high-dose methotrexate, 1733
of mitoxantrone, clinical and pharmacologic studies, 1327

and phase I trial of a daily × 5 schedule of 3-deazauridine, 81

Phase I studies

aclacinomycin A, a new anthracycline antibiotic, in adults with refractory leukemia (phase I–II study), 1619
aclacinomycin A trial, 1127
L-alanosine using a daily × 3 schedule, 73
m-AMSA administered as a continuous infusion (phase I–II study), 1813
bisantrene, clinical evaluation of, 1517
5-day schedule of mitoxantrone (dihydroxyanthracenedione), 1303
5′-deoxy-5-fluorouridine, a new fluoropyrimidine derivative, 1307
DON administered by 5-day courses, 1215
doxorubicin-DNA complex, 2033
5-FU and allopurinol: toxicity modulation and phase II results in colon cancer, 1133
5-FU, doxorubicin, mitomycin, and semustine combination (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas (phase I–II study), 1613
high-dose allopurinol modulation of 5-FU toxicity: trial of an outpatient dose schedule, 1723
indicine-*N*-oxide in patients with advanced cancer, 1509
isotopic immunoglobulin therapy for primary liver cancer: a phase I–II study, 289
methyl-GAG in patients with malignant neoplasms, 65
mitomycin, high-dose, with autologous bone marrow support, 277
PALA and 5-FU, pilot study in patients with advanced cancer, 77
pentamethylmelamine, 1227
and pharmacokinetics of a daily × 5 schedule of 3-deazauridine, 81
13-*cis*-retinoic acid toxicity, 1759
of SOAz, 2039
spirogermanium given by infusion in a multiple-dose schedule, 173
Staphylococcus aureus immunoperfusion, 2027

Phase II studies

aclacinomycin A, a new anthracycline antibiotic, in adults with refractory leukemia (phase I–II study), 1619
AMSA, high-dose, and bone marrow rescue in patients with solid tumors, 385
AMSA in patients with advanced ovarian cancer, 1242
AMSA in patients with metastatic breast cancer: a Cancer and Leukemia Group B study, 1211
AMSA in patients with stage III–IV malignant melanoma, 1237
AMSA, prednisone, and chlorambucil (APC) in the treatment of refractory breast cancer: a Northern California Oncology Group study, 1235
AMSA in previously treated patients with stage III and IV ovarian cancer, 1589
AMSA in prostatic cancer, 208
AMSA in refractory breast cancer, 403
m-AMSA administered as a continuous infusion (phase I–II study), 1813
m-AMSA in disseminated prostatic carcinoma, 1993
m-AMSA in hepatocellular carcinoma: a Southwest Oncology Group study, 1651
anguidine in gastrointestinal malignancies: a Southwest Oncology Group study, 381
aziridinylbenzoquinone (AZQ) in advanced breast cancer: a Cancer and Leukemia Group B trial, 1875
an aziridinylbenzoquinone (AZQ) in disseminated malignant melanoma, 2089
aziridinylbenzoquinone (AZQ) in head and neck cancer, 2097
bisantrene in advanced colorectal cancer: a Cancer and Leukemia Group B study, 1997
bisantrene for advanced hypernephroma, 1653
bisantrene in patients with renal cell carcinoma, 1869
bleomycin, intracavitary, in the management of malignant effusions: a multicenter study, 1903
chlorozotocin in malignant melanoma, breast cancer, and other solid tumors, 1229
chlorozotocin in malignant melanoma: a Southeastern Cancer Study Group report, 1431
cisplatin, maytansine, and chlorozotocin in small cell lung carcinoma (EST 2578), 1417
combination therapy with continuous-infusion PALA and bolus-injection 5-FU, 299
cycloleucine, intermittent high-dose, in colorectal malignancies, 203
cyclophosphamide and prednisolone high-dose regimen in advanced non-Hodgkin's lymphoma of favorable histologic type, 377
deoxynorleucine (DON) in patients with previously treated advanced lung cancer, 1665
dianhydrogalactitol in advanced renal cell carcinoma: a Southwest Oncology Group study, 1231
dianhydrogalactitol (DAG) split-dose intermittent therapy, clinical and pharmacologic evaluation, 283
doxorubicin 4′-epi derivative in patients with advanced colorectal carcinoma, 1757
doxorubicin and mitomycin in non-small cell bronchogenic carcinoma, 177
doxorubicin therapy for advanced islet cell carcinoma, 1567
4′-epi-doxorubicin in metastatic renal cancer, 1219
hydroxy-9-methyl-2-ellipticinium for osseous metastases from breast cancer: a 5-year experience, 1909
etoposide in the management of advanced or recurrent endometrial carcinoma: a Gynecologic Oncology Group study, 1669
5-FU and allopurinol: toxicity modulation and phase II results in colon cancer, 1133
5-FU, doxorubicin, mitomycin, and semustine combination (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas (phase I–II study), 1613
gallium nitrate in breast cancer: a Southwest Oncology Group study, 1591
high-dose megestrol acetate in patients with advanced malignant melanoma, 1239
isotretinoin activity against squamous cell cancers and preneoplastic lesions, 1315
isotopic immunoglobulin therapy for primary liver cancer: a phase I–II study, 289
maytansine in refractory non-Hodgkin's lymphoma: a Southwest Oncology Group study, 1587
medroxyprogesterone acetate (MPA), high-dose, in ad-

vanced chemotherapy-resistant ovarian carcinoma, 407
medroxyprogesterone acetate with tamoxifen in advanced renal cell cancer, 1773
methotrexate and 5-FU use for recurrent head and neck cancer, 1925
methyl-GAG in advanced colon cancer: a phase II trial of the Southwest Oncology Group, 2099
methyl-GAG in the treatment of esophageal carcinoma, 1427
methyl-GAG and vinblastine combination in the treatment of metastatic renal adenocarcinoma, 1585
mitolactol in patients with metastatic melanoma, 195
mitolactol and semustine combination chemotherapy for advanced malignant melanoma, 1425
mitoxantrone in advanced colorectal cancer, 1995
mitoxantrone in previously untreated patients with colorectal adenocarcinoma: a Southwest Oncology Group study, 1779
mitoxantrone trial, 1929
prednimustine in children with recurrent cancer: a Children's Cancer Study Group report, 1583
procarbazine in small cell carcinoma of the lung, 273
randomized comparison of methotrexate with or without L-asparaginase in non-small cell cancer of the lung, 541
razoxane (ICRF-159) in patients with squamous cell carcinoma of the head and neck previously exposed to systemic chemotherapy, 557
results of aggressive chemotherapy for acute nonlymphoblastic leukemia in adults, 1917
sequential methotrexate-5-FU therapy in advanced measurable colorectal cancer, 1563
spirogermanium in advanced epithelial carcinoma of the ovary, 1675
spirogermanium in breast adenocarcinoma: a Cancer and Leukemia Group B study, 1667
tamoxifen in malignant melanoma, 1447
tamoxifen in patients with metastatic malignant melanoma, 1437
thioproline in advanced epidermoid head and neck tumors, 585
vinblastine, bleomycin, and cisplatin combination in advanced malignant melanoma, 567
vinblastine, divided-dose, in non-small cell bronchogenic carcinoma, 171
vindesine in patients with advanced breast cancer, 1729
vindesine for patients with advanced colorectal carcinoma, 583
VM-26 in patients with metastatic transitional cell carcinoma of the urinary tract: an Eastern Cooperative Oncology Group study, 405
Yoshi 864 in epithelial ovarian carcinoma: a Gynecologic Oncology Group study, 1775

Phase III studies
intermittent carmustine (BCNU), cyclophosphamide, and prednisone versus intermittent melphalan and prednisone in myeloma, 1273

Phenylalanine
N-diazoacetyl derivatives of glycine and, antitumor activity against P388 leukemia and B16 melanoma in mice, 179

L-Phenylalanine mustard *see* Melphalan

Phosphoramide mustard
vomiting induced by cyclophosphamide and, in cats, 1625

Prednimustine
in advanced soft tissue sarcomas, 201
in children with recurrent cancer: a Children's Cancer Study Group report, 1583
with methotrexate and 5-FU (PMF): a pilot study, 2081

Prednisolone
and cyclophosphamide, high-dose regimen in advanced non-Hodgkin's lymphoma of favorable histologic type, 377
with vincristine, doxorubicin, and cyclophosphamide (VAP-Cyclo): a short intensive regimen for advanced breast cancer, 1999

Prednisone
with AMSA and chlorambucil (APC) in the treatment of refractory breast cancer: a Northern California Oncology Group study, 1235
with cisplatin, BCNU, and cyclophosphamide in multiple myeloma, 237
with cisplatin and cyclophosphamide, therapy for stage D prostatic cancer, 1403
with cyclophosphamide, doxorubicin, vincristine, and bleomycin, compared with doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine in the treatment of diffuse histiocytic lymphoma, 1279
with cyclophosphamide and vincristine, combination chemotherapy in TNM-classified stage IV mycosis fungoides, 167
with doxorubicin, cyclophosphamide, and carmustine (BCNU) in the treatment of multiple myeloma resistant to melphalan, 451
intermittent with carmustine (BCNU), cyclophosphamide, and, versus intermittent melphalan and prednisone in myeloma, phase III study, 1273
with melphalan, cyclophosphamide, vincristine, and BCNU (M-2 protocol): combination chemotherapy for multiple myeloma, 1971
treatment of advanced plasma cell myeloma with alternate-day, and weekly cyclophosphamide, 1413
vincristine, BCNU, and doxorubicin (VBAP) combination in the treatment of relapsing or resistant multiple myeloma: a Southwest Oncology Group study, 1267

Procarbazine
with CCNU, vincristine, and methotrexate, treatment of relapsed small cell lung carcinoma, 1557
serial analysis of melanoma growth kinetics in a patient receiving bleomycin and: comparison of tumor volume, flow cytometry, and thymidine labeling techniques, 529

Prostatic cancer *see* Cancer, prostatic

Provera *see* Medroxyprogesterone

Public policy
the development of new cancer drugs: a joint venture by the National Cancer Institute and the Food and Drug Administration, 1705

Pyrazofurin
and cytarabine, treatment of refractory acute nonlymphocytic leukemia with a combination of, 1397
and lycurim, action on hepatoma 3924A cells in culture, 343

R

Radiation therapy
- cerebrospinal fluid versus serum concentrations of 5-FU, allopurinol and, during treatment of metastatic brain cancer with 5-FU infusion, allopurinol, and, 571
- chemotherapy combination used prior to, for locally advanced squamous cell carcinoma of the head and neck, 1421
- chemotherapy and radical, combination for primary squamous cell carcinoma of the anal canal, 489
- combination carmustine (BCNU) and dianhydrogalactitol in the treatment of primary brain tumors recurring after, 1647
- and combined chemotherapy with doxorubicin, bleomycin, vinblastine, and dacarbazine for advanced lymphoepithelioma, 1207
- combined with intra-arterial 5-FU chemotherapy for treatment of localized adenocarcinoma of the pancreas: a Southwest Oncology Group study, 549
- versus combined modality treatment of stage I and II Hodgkin's disease, 799
- doxorubicin with or without, and with or without levamisole immunotherapy in limited squamous cell carcinoma of the lung: a Southwest Oncology Group randomized study, 1113
- with doxorubicin in malignant pleural mesothelioma, 1605
- essential thrombocythemia: response during first year of therapy with radioactive phosphorus and melphalan: a Polycythemia Vera Study Group report, 1495
- with 5-FU infusion and allopurinol, 571
- involved-field versus extended-field radiotherapy versus involved-field radiotherapy followed by six cycles of MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) in stage I and II Hodgkin's disease, 789
- isotopic immunoglobulin therapy for primary liver cancer: a phase I–II study, 289
- late effects of, in the treatment of Hodgkin's disease, 991
- long-term cardiovascular evaluation of patients with Hodgkin's disease treated by thoracic mantle irradiation, 1003
- multidisciplinary treatment of advanced Hodgkin's disease by an alternating chemotherapeutic regimen of MOPP/ABVD and low-dose radiation therapy restricted to originally bulky disease, 907
- results in 15 patients with primary non-Hodgkin's lymphoma of bone treated by systemic chemotherapy combined with, 1859
- results of lung irradiation for patients with large mediastinal masses and/or hilar disease, 819
- role in the treatment of Hodgkin's disease, 915
- treatment results in children with Hodgkin's disease using MOPP (mechlorethamine, vincristine, prednisone, and procarbazine) and low-dose, extended-field, 949
- upper hemibody and local chest irradiation as consolidation following response to high-dose induction chemotherapy for small cell bronchogenic carcinoma—a pilot study, 1609

Radioimmunoassay
- for peplomycin, 1365

Razoxane
- in patients with squamous cell carcinoma of the head and neck previously exposed to systemic chemotherapy, 557
- studies of Baker's antifol, methotrexate and, in advanced gastric cancer: a Gastrointestinal Tumor Study Group report, 1713

Renal cell cancer *see* Cancer, renal cell

13-*cis*-Retinoic acid
- toxicity of: a phase I study, 1759

Rubidazone *see* Zorubicin

Rubidomycin *see* Daunorubicin

S

Scopolamine
- versus placebo administered by transdermal patch for the control of cisplatin-induced emesis: results of a randomized double-blind crossover trial, 1975

Semustine
- with 5-FU, doxorubicin, and mitomycin (FAMMe) in the treatment of adenocarcinoma of the stomach, gastroesophageal junction, and pancreas: phase I–II study, 1613
- and mitolactol combination chemotherapy for advanced malignant melanoma: a phase II study, 1425

SOAz
- a phase I study of, 2039

Soft tissue cancer *see* Cancer, soft tissue

Spirogermanium
- in advanced epithelial carcinoma of the ovary: a phase II study, 1675
- in breast adenocarcinoma: a Cancer and Leukemia Group B phase II study, 1667
- by infusion in a multiple-dose trial: a phase I study, 173

***Staphylococcus aureus* Cowan I**
- immunoperfusion with: a phase I trial, 2027

Statistics
- guidelines for medical research reports, 217

Steroids
- effective control of cisplatin-induced nausea using droperidol and high-dose, 243

Streptozocin
- pharmacology and toxicity of ip, in ovarian cancer: a case study, 1595
- review of its pharmacology, efficacy, and toxicity, 427

T

Tamoxifen
- in malignant melanoma: a phase II study, 1447
- with medroxyprogesterone acetate in advanced renal cell cancer: a phase II study, 1773
- in metastatic melanoma, Southwest Oncology Group experience with, 1680
- before and after orchiectomy in advanced male breast cancer, 1882
- in patients with metastatic malignant melanoma: a phase II study, 1437
- response to oophorectomy after failure of, in a premenopausal patient, 1869
- in the treatment of metastatic malignant melanoma, 1771
- trial at a 40-mg daily dose after disease progression during therapy at a 20-mg daily dose, 1445

Taxol
tubulin-dependent biochemical assay for, and application to measurement of the drug in serum, 1381
T-cell cancer *see* Cancer, T-cell
Teniposide (VM-26)
combination chemotherapy for patients with relapsed malignant lymphoma using methyl-GAG and, 1121
comparative trial of two combination chemotherapy regimens based on, for the treatment of advanced malignant lymphomas, 2021
in patients with metastatic transitional cell carcinoma of the urinary tract: an Eastern Cooperative Oncology Group phase II study, 405
vincristine and, comparison in chemotherapy for non-Hodgkin's lymphoma, 43
Testicular cancer *see* Cancer, testicular
6-Thioguanine
response of hypereosinophilic syndrome to cytarabine and, 545
Thioproline
in advanced epidermoid head and neck tumors: a phase II trial, 585
Thiotepa
treatment of neoplastic meningitis with intraventricular methotrexate and, 1549
Thrombocythemia
essential (ET): response during first year of therapy with melphalan and radioactive phosphorus: a Polycythemia Vera Study Group report, 1495
Thymidine
comparison of protection from methotrexate toxicity by folinic acid and, in human lymphoid cell lines, 2051
Triazinate *see* Baker's antifol
3,4,5-Trihydroxybenzohydroxamic acid (VF-122)
cytotoxic and cell kinetic effects of, in hepatoma 3924A cells, 2071
Tubercidin
comparison of the effects on cultured L1210 leukemia cells of the ribosyl, 2′-deoxyribosyl, and xylosyl homologs and adenosine and, alone or in combination with 2′-deoxycoformycin, 317
Tuberculosis
possible relationship between previous, and long-term survival after solid tumor chemotherapy, 206
Tubulin
-dependent biochemical assay for taxol and application to measurement of the drug in serum, 1381
Tumor systems, animals
B16 melanoma
antitumor activity of *N*-diazoacetyl derivatives of glycine and phenylalanine against mouse P388 leukemia and, 179
C1300 mouse neuroblastoma
effects of interferon on, 1575
colon adenocarcinoma xenografts
combinations of 5-FU, hypoxanthine, and allopurinol in chemotherapy for human, 1201
Dunning R3327H prostatic adenocarcinoma
response to radiation and various chemotherapeutic drugs, 1837
Friend murine leukemia virus
hematopoietic stem cells in infected mice undergoing chemotherapy: effects of 5-FU, 1949
human tumor xenografts
ultrasonic hyperthermia and drugs in the therapy for, 1635
L1210 leukemia
antitumor activity and toxicity of cisplatin analogs, 135
combination chemotherapy with cyclophosphamide and ftorafur against advanced murine, 1631
leukemia, acute
chemoimmunotherapy for autochthonous, in rat, 391
Lewis lung carcinoma
prophylactic antimetastatic treatment with aryldimethyltriazenes as adjuvants to surgical tumor removal in mice bearing, 115
M5075/74A ovarian cancer
hexamethylmelamine and pentamethylmelamine tissue distribution in mice bearing, 127
M5076/73A ovarian reticular cell sarcoma of the mouse
activity of 1-*p*-(3,3-dimethyl-1-triazeno) benzoic acid potassium salt in, 1945
murine leukemias and solid tumors
vinylogous carbinolamine tumor inhibitors, activity of bis(acyloxymethyl) derivatives of pyrroles and pyrrolizines against a panel of, 91
P388 leukemia
antitumor acitivity of *N*-diazoacetyl derivatives of glycine and phenylalanine against mouse B16 melanoma and, 179
9L rat brain tumor
combination chemotherapy for, 575
rat glioma
evaluation of the lipid-soluble diaminopyrimidines, metoprine and etoprine, in the avian sarcoma virus model of, 99
3924A rat hepatoma
cytotoxic and cell kinetic effects of 3,4,5-trihydroxybenzohydroxamic acid (VF-122) in, 2071
transplantable tumors of mice
response to anthracenedione derivatives alone and in combination with clinically useful agents, 1187

U

Urinary tract cancer *see* Cancer, urinary tract

V

VAB-3
combination chemotherapy in primary mediastinal germ cell tumors, 1405
VAP-Cyclo (vincristine, doxorubicin, prednisolone, and cyclophosphamide)
a short intensive regimen of chemotherapy with, for advanced breast cancer, 1999
Velban *see* Vinblastine
Vinblastine
with bleomycin and cisplatin, treatment of advanced malig-

nant melanoma with, 1299
combination with bleomycin and cisplatin in advanced malignant melanoma: a phase II study, 567
combined chemotherapy with doxorubicin, bleomycin, and dacarbazine, and radiotherapy for advanced lymphoepithelioma, 1207
divided dose in non-small cell bronchogenic carcinoma: a phase II study, 171
failure of 5-day infusion in the treatment of patients with advanced refractory breast cancer, 1783
and methyl-GAG combination in the treatment of metastatic renal adenocarcinoma, 1585
with mitomycin and carmustine (BCNU) as third-line chemotherapy in refractory breast carcinoma: a pilot study, 559
with vincristine and vindesine, effect on neutrophil phagocytosis and iodination, 505

Vincristine
acute vascular episodes associated with therapy for carcinomas of the upper aerodigestive tract with bleomycin, cisplatin, and, 369
BCNU, doxorubicin, and prednisone (VBAP) combination in the treatment of relapsing or resistant multiple myeloma: a Southwest Oncology Group study, 1267
with bleomycin, cisplatin, and methotrexate in advanced non-small cell bronchogenic carcinoma, 205
with carmustine (BCNU) and bleomycin in patients with squamous cell bronchial carcinoma resistant to cyclophosphamide, doxorubicin, methotrexate, and procarbazine (CAMP), 1877
with CCNU, methotrexate, and procarbazine, treatment of relapsed small cell lung carcinoma, 1557
with cyclophosphamide, doxorubicin, prednisone, and bleomycin, compared with doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine in the treatment of diffuse histiocytic lymphoma, 1279
with cyclophosphamide and prednisone (CVP), combination chemotherapy in TNM-classified stage IV mycosis fungoides, 167
with doxorubicin, cyclophosphamide, methotrexate with leucovorin rescue, and cytarabine, compared with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin in the treatment of diffuse histiocytic lymphoma, 1279
with doxorubicin, prednisolone, and cyclophosphamide (VAP-Cyclo): a short intensive regimen for advanced breast cancer, 1999
-doxorubicin therapy for Wilms' tumor: invited comment on the Milwaukee pilot study, 1795
-doxorubicin therapy for Wilms' tumor: a pilot study, 1791
massive overdose of: failure of leucovorin to reduce toxicity, 1967
with melphalan, prednisone, cyclophosphamide, and BCNU (M-2 protocol): combination chemotherapy for multiple myeloma, 1971
teniposide and, comparison in chemotherapy for non-Hodgkin's lymphoma, 43
with vinblastine and vindesine, effect on neutrophil phagocytosis and iodination, 505

Vindesine
-associated angina and ECG changes, 587
with cisplatin, doxorubicin, and cyclophosphamide, combination chemotherapy for non-small cell lung cancer, 247
an effective agent in the treatment of non-small cell lung cancer, 1709
with 5-FU and mitomycin (FEMi) in the treatment of extensive adenocarcinoma and large cell undifferentiated carcinoma of the lung: a Southwest Oncology Group study, 553
with hexamethylmelamine and mitomycin as second-line treatment of small cell cancer of the lung, 1873
monochemotherapy for non-small cell lung cancer: a report of 45 cases, 1409
overdose of, 1247
in patients with advanced breast cancer: a phase II study, 1729
for patients with advanced colorectal carcinoma: a phase II evaluation, 583
in the treatment of squamous cell carcinoma, adenocarcinoma, and large cell carcinoma of the lung, 305
with vincristine and vinblastine, effect on neutrophil phagocytosis and iodination, 505

VM-26 *see* Teniposide

VP-16-213 *see* Etoposide

W

Wilms' tumor *see* Cancer, Wilms' tumor

Y

Yoshi 864
in epithelial ovarian carcinoma: a Gynecologic Oncology Group phase II clinical study, 1775
and medroxyprogesterone acetate in adenocarcinoma of the kidney: a Southwest Oncology Group study, 1781

Z

Zinostatin
severe toxicity and death associated with administration of, 410

Zorubicin
and 5-azacitidine for patients with previously treated acute nonlymphocytic leukemia: a Cancer and Leukemia Group B pilot study, 563

Author Index

A

Aabo K, 273, 407
Abe M, 1645
Abele R, 1307
Abeloff MD, 457, 1601, 1880
Abrams S, 171
Abramson N, 1273
Adair S, 1791
Adjizian JC, 1983
Adolphson CC, 209
Advani SH, 1169
Aherne GW, 1365
Ahmann DL, 567, 1239, 1425, 2089
Aisner J, 221, 1127, 1237, 1557, 1773, 1875
Ajani JA, 1227, 1987
Al-Sarraf M, 31, 471, 1993
Alberto P, 1307
Alberts DS, 1315
Alexanian R, 1267
Alison RE, 717
Altman SJ, 1781
Amare M, 1257, 1267
Ambrus JL, 201
Ames MM, 283, 1579
Amrein PC, 1211
Anbar D, 1997
Anderson J, 2021
Anderson JL, 493
Anderson KC, 1929
Anderson RM, 259
Anderson T, 1229, 1975
Anderson WK, 91
Andrysiak T, 589
Anku V, 2106
Anson N, 1885
Appelbaum FR, 1397, 1863
Applefield MM, 1003, 1127
Ardman B, 1785
Arlin ZA, 907, 1479, 1619
Armitage JO, 1917
Arnold AM, 1719
Arnold S, 147, 157
Arnon R, 107
Arseneau JC, 1285
Arthur D, 1683
Ashford RFU, 1884
Aunan SB, 1917
Austerlitz J, 1879

B

Bacci C, 1859
Bachur NR, 339
Bae E, 1219
Bains M, 1427
Bainton DF, 609
Bajetta E, 1299
Bakemeier RR, 855
Baker LH, 203, 371, 1231, 1447, 1591, 1673
Balcerzak SP, 253
Baldini L, 179
Balducci L, 31, 1231
Ballin A, 2000
Ban J, 343, 2071
Band PR, 1759
Barbieri A, 585
Barlock AL, 1229, 1767
Barlogie B, 529
Barnes JM, 855
Bartolucci A, 199, 899
Baum ES, 1583
Bean HA, 717
Beard R, 1635
Beckstead JH, 609
Bedikian A, 1517
Bell R, 377
Bellet RE, 557
Benjamin RS, 2033
Bennett JM, 855, 1273, 2021
Bennetts RW, 1613
Beran M, 385
Berard CW, 653, 2021
Berenberger J, 410
Bergerat JP, 1983
Bergsagel DE, 717
Berk PD, 1495
Bernhardt B, 1211
Bernstein A, 107
Berry J, 1403
Berry JP, 1529
Bertino JR, 871, 1279
Bertoli G, 115
Bertoni F, 1859
Besner JG, 1759
Betsill WL Jr, 1521
Bhathena BD, 371
Bhuchar VK, 375
Bianco AR, 1435

Bigner DD, 99
Bjorkholm M, 701
Blessing JA, 1669, 1775
Blitt CD, 259
Bliven SF, 1545
Block ER, 359
Block JB, 121
Bloomfield CD, 563, 819, 835, 889
Blumenschein GR, 385, 1517, 1641
Boccardo F, 585
Bodey GP, 73, 385, 1227, 1517, 1641, 1991, 2033
Boesen E, 2081
Bogliolo G, 2061
Bolis G, 1233
Bollag W, 1307, 1375
Bonadonna G, 403, 881, 1299
Bond WH, 2005
Bonfante V, 881
Bonnet JD, 1267, 1587, 1781
Bonnem EM, 1661, 1995
Bonomi PD, 1417
Borch RF, 1889, 1933
Borison HL, 363, 1625
Børmer O, 1437
Borowitz MJ, 667
Bottomley R, 381, 1267
Bowles C, 2027
Bowman D, 1553
Braat PC, 1967
Bradner WT, 135, 187, 1831
Bramwell VHC, 1997
Brandes LJ, 1413
Bratt PM, 1889
Breithaupt H, 1733
Briere J, 1495
Brille P, 1529
Brindley C, 1957
Brodovsky HS, 2021
Broggini M, 127
Bronson RT, 1349
Brooks R, 1315
Broun GO Jr, 237, 1409
Browman GP, 2051
Brown TC, 717
Bruckner HW, 65, 1713
Brunet R, 1563
Brunner KW, 451, 1561, 1873
Bruno S, 81
Buckner CD, 1463
Budillon G, 1435
Budman DR, 171, 173, 1667, 1675, 1875
Bui NB, 2107
Bukowski RM, 381, 1651
Bunn PA, 1223
Burgert EO Jr, 961
Burgess MA, 73, 1987, 2033
Burns CP, 1917
Burns D, 1431
Burrichter H, 615
Bush RS, 717
Busutti L, 1763
Butler JJ, 789
Buzzoni R, 1299
Byfield JE, 571
Byrne GE, 645

C

Cabanillas FF, 1227
Cadman EC, 1279
Cahn JY, 1983
Calanog A, 1809
Calderoni P, 1859
Callahan K, 2097
Camacho F, 1809
Camaggi CM, 1819, 2043
Camitta B, 1791
Campanacci M, 1859
Campbell TN, 1723
Canellos GP, 1015, 1585
Canobbio L, 585
Canova N, 1819
Cantrell JE, Jr, 1563
Capizzi RL, 541
Capuano G, 1435
Carbone A, 167, 1207, 1971
Carlin DA, 553
Carney DN, 1223
Carpenter JT, Jr, 209
Carpentier Y, 2039
Carrera CJ, 2085
Carter SK, 163
Casper E, 247
Cass CE, 317
Castellino RA, 697
Catalano RB, 243, 557, 1609
Cattan A, 2039
Chahinian AP, 1291
Champion LAA, 2013
Champlin R, 277
Chan H, 949
Chang C-P, 91
Chapman JD, 1837
Chapman R, 1427
Charcosset J-Y, 327
Cheever MA, 1397
Chello PL, 351
Chen M, 1279
Chen T, 381, 937, 1113, 1779
Cheng E, 247
Chenoweth L, 2005
Chin HW, 211
Chlebowski RT, 121
Chohan V, 505
Chou T-C, 1479
Chua T, 717
Chuang V, 1501
Clark RA, 2108
Clark RM, 717
Clarkson BD, 907

Clift RA, 1463
Coates AS, 19, 177, 1242
Cobleigh MA, 1709
Cocconi G, 1263
Cohen GI, 1929
Cohen HJ, 237
Cohen MH, 1223
Colby TV, 675
Coldman AJ, 439
Coleman M, 410, 563, 835, 1211, 1667
Coleman MS, 253
Collins JM, 157
Collins RD, 1107
Colombo N, 1233, 1681
Colombo T, 127, 1945
Coltman CA, Jr, 847, 1023, 1689
Colvin M, 1487, 1625
Comis R, 410
Condon RE, 1357
Coninx P, 2039
Cooper MR, 419, 889, 1035
Coral FS, 1455
Corbett TH, 1187
Corbitt R, 2027
Corey PF, 91
Cornwell GC, III, 475
Cortes E, 1211
Costanti B, 2043
Costanzi J, 1680
Costello W, 855
Couette JE, 1909
Coveney JR, 1889
Cowan JD, 1779
Cowens JW, 509
Cox RS, 743
Crain SM, 2097
Creagan ET, 567, 1239, 1425, 1665, 2089
Creaven PJ, 81, 509
Creech RB, 1609
Creech RH, 557, 1417
Crivellari D, 559, 1207, 1971
Croker BP, 667
Crout JR, 1705
Crowther D, 1771, 1997
Cruz AB Jr, 549
Cullinan SA, 2093
Cummings BJ, 489, 1421
Cummings G, 203, 371
Cundiff D, 121
Cunningham J, 809, 1015
Curran J, 1387
Cuttner J, 410

D

D'Amore F, 2061
D'Angio G, 1795
D'Incalci M, 121, 131, 1233, 1681, 1945
D'Souza BJ, 2013
Dabich L, 751
Dalley D, 717
Dambra J, 243
Damle SR, 1169
Daubenspeck JA, 363
Davis F, 633
Davis HL, 1215
Davis TE, 1285
Dawes PJDK, 581
Dean D, 2027
Dean JC, 259
DeGregorio MW, 2085
Deisseroth A, 2027
De Lena M, 403
Delozier T, 1909
De Mûelenaere G, 1605
De Sousa M, 969
DeBellis R, 267
DeBoer G, 717
Decker DA, 1993
DeGraw JI, 351
DeLisi V, 1263
DePersio EJ, 847
DeVita VT, 653, 925
DeWys WD, 1387
Degnan TJ, 171, 173, 1675
Delaney D, 541
Delgado G, 1669, 1775
Denefrio J, 1439
Denes AE, 199
Denning B, 1729
de Palo G, 1681
Deprez-DeCampaneere D, 395
Desai MH, 1371
Deschamps M, 1759
Dhabuwala CB, 1993
Di Blasio, 1263
Dick FR, 1917
Dicke DA, 385
Dicke KA, 1991
Didolkar MS, 201
Diehl V, 615
Dietrich MF, 121
Dillman RO, 1399
Dillon MB, 1981
Di Marco AR, 2043
Dinapoli RP, 1647
Dindogru A, 203
Diorio G, 1759
Dixon DO, 847, 1023, 1267
Dombernowsky P, 273, 305, 407
Donaldson SS, 977
Donovan PB, 1495
Dorfman RF, 675
Dorick DM, 351
Dorreen MA, 759
Dorval T, 1803
Dosik GM, 73
Dosik H, 475
Dougherty WJ, 1139
Dowling R, 2027

Drelichman A, 471, 1993
Drewinko B, 529
Dukeman M, 1427
Dupont JC, 1596
Durant JR, 899

E

Eagan RT, 283, 1647, 1665
Eakin DL, 545
Earhart RH, 1215
Einhorn LH, 15, 1709, 2005
Einhorn N, 1285
Eisenberger MA, 1439
El Dareer SM, 183
Elford HL, 1825
Elfring GL, 1333
Elliott FG, 1837
Elson PJ, 405
Engelking C, 1797
Engstrom PF, 557
Ensminger WD, 493
Erlichman C, 489, 1783
Ervin TJ, 299
Ettinger DS, 289, 1417, 1487, 1981
Evans RC, 961
Everson LK, 2093
Eyre HJ, 1651

F

Faanes RB, 535
Fabian CJ, 1591, 1673, 1680, 2099
Faguet GB, 1495
Falkson G, 1605
Farber LR, 871, 1279
Farina P, 231
Farradji A, 1983
Faye L, 1653
Fearon F, 463
Fefer A, 1397
Ferrazzi E, 1274
Fetting JH, 363, 1487, 1601, 1625
Fine S, 489
Fiorentino MV, 1247
Fisher R, 937
Fisher RD, 25
Fisher RI, 681, 1229
Fixler H, 1349
Fleit JP, 1797
Fleming TR, 1101
Flesch M, 1983
Fletcher WS, 31, 1673
Flexner JM, 733
Florczyk AP, 187, 1831
Floyd RA, 571
Folstein MF, 1487
Fonatsch C, 615
Fontana J, 1729
Foon KA, 1241
Forastiere AA, 1875, 1997, 2097
Ford J, 377
Ford RJ, 633
Fossa SD, 1219
Fosser V, 1247
Fox RM, 19, 177, 1242
Fraass BA, 991
Frankel S, 571
Freedman M, 949
Frei E III, 1455
Freireich EJ, 2033
Friedman MA, 163, 1235
Friedman RD, 339
Fritze D, 1785
Frondoza CG, 1535
Frustaci S, 1207
Fryer C, 937
Frytak S, 1665
Fuks JZ, 1237, 1773
Fuller LM, 775, 789, 937
Furnas BE, 1709
Furner R, 147

G

Gad-el-Mawla N, 1587
Gadgil SD, 1169
Gale RP, 277, 589
Gallagher CJ, 377
Galle P, 1529
Galligioni E, 167, 559, 1207, 1877, 1882
Gams RA, 899
Garattini S, 231, 1945
Garay GE, 1596
Garbe E, 2039
Garnick MB, 1585, 1929
Gasser AB, 1561
Gaynon PS, 1583
Geddes DM, 463
Gee TS, 907, 1479
Gehan E, 937, 1089
Gelber RD, 1594
Gelinas M, 1759
Gerdes J, 615
Germano G, 1307
Gerretson L, 163
Gescher A, 1957
Gherlinzoni F, 1859
Ghio R, 2061
Gilchrist GS, 937, 961
Gill DP, 545
Gill S, 1825
Gilmartin E, 1315
Ginsberg S, 1211, 1667
Giovannini M, 1819, 2043
Giraldi T, 115, 179, 1751
Girard C, 1759
Glass EC, 1683

Glassberg A, 163
Glatstein E, 11, 991, 1743, 2027
Glick JH, 855, 2021
Glicklich M, 1791
Glicksman AS, 835, 1035, 1545
Glidewell OJ, 410, 835, 889
Godfrey TE, 25
Golbey R, 247, 1405, 1427
Goldberg JD, 1291, 1495
Goldhirsch A, 1873
Goldie JH, 439
Goldschmidt E, 1803
Goldstein L, 410
Golomb HM, 733, 827
Gomez GA, 43
Good RA, 969
Goodman GE, 751
Goodwin JW, 1257
Goold MA, 1884
Gordon G, 1145
Gormley P, 1813
Gorshein D, 809
Gospodarowicz M, 717
Gottlieb AJ, 563, 835, 889, 1035
Goudsmit, R, 1967
Gouveia Y, 1529
Gralla RJ, 247, 2091, 2108
Gramellini M, 1763
Grant C, 1501
Grant JM, 19
Gray DP, 253
Green JA, 1719
Green MR, 1211, 1399
Green SJ, 567, 1239, 1425, 2089
Greenberg BR, 163
Greenberg M, 949
Greenberg MS, 563
Greenberg PD, 1397
Greenspan EM, 206
Greenwald, E, 1449
Grever MR, 253
Gribbin M, 949
Griffin JP, 1321
Griffiths JC, 571
Grigoletto E, 167, 559, 1207, 1877, 1971
Griswold DP Jr, 1187
Grochow LB, 1487
Grogan TM 653
Groover RV, 1647
Grossie VB Jr, 85, 1631
Grossman B, 243
Grossman SA, 1549
Grozea PN, 553, 847
Grumbine FC, 1981
Guarino AM, 147, 157
Guarneri D, 585
Gudauskas GA, 439
Guimaraes M, 463
Gutensohn NM, 689
Gutierrez PL, 339

H

Hacker MP, 1845
Hagemeister FB, 789
Hahn RG, 1567
Halat MJ, 91
Halberg F, 1933
Hald I, 407
Hall C, 1145
Hall SW, 2033
Halsall GM, 1903
Hamel E, 1381
Hammond D, 399, 1583
Han T, 43
Hankey BF, 653
Hanks GE, 805
Hannigan J, Jr, 163
Hansen HH, 273, 305
Hanson J, 1667
Hanson W, 937
Harper PG, 463
Harpur ES, 1957
Hart RD, 65
Harvey HA, 1303
Harwood AR, 489
Haskell CM, 1241
Hasselback R, 717
Haut A, 1267
Hayat M, 1803
Haynie TP, III, 1501
Hays D, 937
Heelan R, 1427
Heifetz LJ, 1613
Heintz F, 615
Heintz R, 1307
Heit W, 615
Heller R, 937
Hellman S, 733, 809, 915
Henderson BM, 1139
Henderson E, 43, 81, 451
Henderson IC, 1349
Henderson SD, 575
Hendrickson CA, 163
Henle W, 1387
Henry PH, 451
Herman TS, 259
Hermann RC Jr, 1647
Hernandez E, 1981
Heron JF, 1909
Herring DF, 805
Herrmann R, 1785
Herve P, 1983
Hewlett JS, 31
Heyn R, 399
Higgins G, 937
Hill DL, 183
Hiramoto RN, 237
Ho DHW, 85
Hobson AS, 1521
Hoerni B, 2107

Hoeroff R, 1343
Holcenberg JS, 387, 2105
Holland JF, 65, 451, 835, 889, 1291, 1509
Holm G, 701
Holohan T, 2027
Holyoke ED, 201
Hong CB, 1845
Hood CI, 359
Hoogstraten B, 1231
Hopkins HA, 1521
Hoppe RT, 743
Hørbov S, 305
Hornbeck CL, 571
Horton J, 1273
Hoschner J, 471
Houchens D, 1635
Houghton JA, 1201
Houghton PJ, 1201
House BA, 1723
Howell A, 1997
Howell SB, 1133, 1723, 1825
Howerd J, 1591
Howser D, 1975
Hrushesky WJM, 1933
Hubbard SM, 1, 1975
Huftalen JB, 135
Humphrey RL, 1535
Hutchison GB, 775
Hutter JJ, 259, 593
Hynes HE, 1591

I

Iafelice G, 1819
Iaffaioli RV, 1435
Iatropoulos MJ, 1145
Ichiba K, 1645
Ignoffo R, 1885
Ihde DC, 1223
Ilstrup DM, 961
Ingle JN, 1665
Israels LG, 1413

J

Jacobs C, 1925
Jacobs J, 471
Jacquemin-Sablon A, 327
Jaenke R, 395
Jaffrey IS, 1291
James S, 1365
James VC, 1139
Jasmin C, 1803
Jenkin D, 937, 949
Jezekova D, 2039
Johansson B, 701
Johns DG, 1381
Johnson DE, 57, 1501
Johnson G, 1387
Johnsson J-E, 1441
Johnston DA, 385, 789
Johnston-Early A, 1223
Jones AE, 759
Jones AN, 91
Jones FE, 1357
Jones RD, 1613
Jones SE, 645, 751, 847
Joss RA, 1873
Juret P, 1909

K

Kadin ME, 601
Kalman AM, 1867
Kamen BA, 2105
Kant JA, 653
Kaplan BH, 1285
Kaplan HG, 1397
Kaplan HS, 743, 977
Kapp DS, 871
Karakousis CP, 201
Karlin DA, 1613
Kasdorf H, 267
Keane TJ, 489
Kearsley JH, 1242
Keating MJ, 1991
Kefford RF, 19
Kelsen DP, 247, 1427, 2091
Kemeny N, 1757
Kempin SJ, 907, 1619
Kende G, 2000
Kennedy BJ, 1683, 1933
Khan AB, 2013
Khandekar JD, 405, 1244
Khato J, 517
Khoo RKK, 1977
Khwaja TA, 1853
Kiang D, 1211
Kidowaki T, 1575
Kimler BF, 575
King M, 1783
Kinsella TJ, 421, 991
Kinzie JJ, 471, 805
Kirchner HH, 615
Kirkwood JM, 195
Kirsch M, 541
Kish J, 471
Kishida T, 1575
Klein JL, 289, 1981
Klepp O, 1437
Klock JC, 2085
Knight WA, III, 2099
Knospe WH, 1495
Knost JA, 1729
Kochwa S, 475
Koeller JM, 1215
Kohler M, 1235
Koretz MM, 1475, 1885

Kosloff C, 247
Kosunoki T, 1575
Kovach JS, 283, 1579
Koziner B, 907
Krakoff IH, 1321, 1327
Kramer S, 805
Kraut EH, 1673
Kreja L, 1949
Krivit W, 399, 1583
Kroener JF, 1133
Krook JE, 2093
Kuenzlen E, 1723
Kufe DW, 299
Kuhn JG, 1869
Kukla LJ, 369
Kun L, 1791
Kung F, 937
Kuraishi Y, 1645
Kyle RA, 451

L

Lad T, 369
Lagarde C, 1563
Lakey WH, 1837
Landaw SA, 1495
Lane WW, 197
Lange B, 1699
Langston CM, 1881
Lanzotti VJ, 375
Larochelle D, 1759
Larson DF, 259
Larson RA, 767
Lasnitzki I, 1375
Lassiani L, 1751, 1945
Laster WR Jr, 1187
Laszlo J, 1495
Lavin PT, 1567
Lawrence HJ, 1475
Lawrence J, 1885
Lawrence R, 871
Layton DD, Jr, 1647
Lazarus P, 1595
Lea JW, Jr, 1399
Leavitt J, 1409
Leclaire R, 1759
Lee BJ, 907
Lee CKK, 819
Lee KL, 1077
Lee YJ, 31
Legha S, 385, 1651, 1991
Lehar TJ, 1399
Lehotay DC, 311
Leichman CG, 1447
Leichman L, 203, 549, 2099
Leichner PK, 289
Leith JT. 1545
LeRoy AF, 1529
Lessner LE, 1439
Lester R, 2027
Le Talaer JY, 1909
Leventhal BG, 2013
Levey BA, 311
Levey GS, 311
Levi F, 1933
Levi JA, 177, 1242
Levi-Schaffer F, 107
Levin J, 1449
Levine AS, 1391, 1699
Levine NS, 1315
Levitt M, 1237, 1553
Levitt SH, 819
Lewis JP, 1475
Lien HH, 1219
Lin CM, 1381
Lippman ME, 1229
Lipton A, 1303
Lister TA, 377, 759
Litterst CL, 147, 157, 1661
Lloyd HH, 183
Loesch DM, 2099
Logothetis CJ, 57, 1501
Loh J, 471
Lokich JJ, 1713
Longo DL, 925, 1975
Loo TL, 85, 1631
Looney WB, 1521
Loutfi A, 409
Low JE, 1889
Lowe MC, 157, 1571
Lowenthal RM, 505
Luedke DW, 1409
Luedke SL, 1409
Luna MA, 2033
Lurie P, 1273

M

Macbeth FR, 1719
Macdonald JS, 1
Machover D, 1803
MacKay IS, 1884
Madajewicz S, 197
Mador D, 1837
Magri MD, 559, 1207, 1877
Mahal PS, 1613
Maizel AL, 633
Makuch RW, 217, 1743
Malone JM, 259
Maloney TR, 1257
Malpas JS, 377, 759
Mandel EM, 1291
Manegold C, 1785
Mangiacasale D, 483
Mangioni C, 231, 1233
Maral R, 1803
March P, 915
Marcus F, 1235

Margolese R, 1595
Marit G, 2107
Markman M, 1880
Marks V, 1365
Marraro D, 1819
Marsh JC, 499
Martini N, 1405
Martoni A, 1763, 1819
Mason BA, 243, 1609
Mass R, 1587
Massa G, 2061
Mathé G, 1529, 1803
Mattox D, 2097
Mauch P, 733, 809, 1015
Mayer RJ, 1455
McCarthy LE, 363, 1625
McClure P, 949
McCormack JJ, 1321, 1327
McCormack K, 2027
McCracken JD, 31, 549, 1779
McCredie KB, 1991
McCullough DC, 2013
McDonald B, 371, 1779
McDonald RN, 1403
McGovren JP, 1333
McGuire WP, 369
McGunagle D, 147
McIntyre OR, 451, 475
McKee MJ, 1845
McKinney DR, 583
McManus ME, 1343
McPhee MS, 1837
McPherson HL Jr, 91
Mead GM, 1719
Medina W, 195
Meeker B, 1837
Meguro S, 1645
Mehta S, 633
Mellstedt H, 701
Mendelsohn J, 1399
Merlano M, 585
Merluzzi VJ, 535
Merrill JM, 2021
Meshad MW, 299
Meshorer A, 107
Messerschmidt G, 2027
Metzgar RS, 667
Meyskens FL, 593, 1315
Micetich KC, 1813
Michaelson R, 1757
Michalski B, 1803
Mietlowski WL, 1273
Mikouchi S, 1645
Miliauskas JR, 653
Miller CF, 1678
Miller DR, 483
Miller HJ, 1244
Miller TP, 553, 645
Minna JD, 1223
Minnhaar G, 385
Minton MJ, 1445
Mira J, 1113
Misset JL, 1803
Mitchell EP, 1995
Mittelman A, 81
Moayeri H, 43
Moccio DM, 351
Moertel CG, 77, 1567
Moore R, 1837
Morag B, 2000
Morantz RA, 575
Morrison A, 410, 1211
Morrison FS, 1257
Mouridsen HT, 2081
Mughal T, 1884
Muhs WH, 317
Muir W, 211
Murphy S, 1495
Murray K, 1549
Muss H, 1775
Muzzulini C, 2061
Myers J, 907
Myers JW, 1869

N

Naeher C, 43
Nasca S, 2039
Natale RB, 208, 2091
Nawabi IU, 451
Neefe J, 1995
Neely SM, 827
Neijstrom E, 541
Neiman RS, 855, 2021
Nelson JC, 25
Nerenberg MI, 1279
Nettesheim KM, 1601
New JS, 91
Newcome SR, 751
Newcomer LN, 1279
Newman RA, 1321
Ng RP, 1977
Nichol CA, 99
Nichols WC, 1665
Nicolau G, 1173
Nines R, 1635
Nirenberg A, 1687
Nisce LZ, 907
Nisi C, 115, 1751
Nissen NI, 799, 835, 889, 1035
Noble JF, 1139, 1145
Nordentoft AM, 799
Nordlund M, 871
North L, 789

O

O'Bryan RM, 1113, 1651, 2099
O'Connell MJ, 77, 2021
O'Donnell JF, 410

Occhipinti SJ, 1159
Ochoa M Jr, 1591
Oechler H, 1791
Ogenstad S, 701
Ohnuma T, 65, 1509
Oishi N, 31, 549, 1593
Oku T, 1575
Olah E, 343, 2071
Olson M, 549
Omura GA, 591
Order SE, 289, 1981
Orlow EL, 855
Osborne L, 2027
Østerlind K, 305
Ostrowski MJ, 1903
Ovejera A, 1635
Ozols RF, 1767

P

Paccagnella A, 1247
Padilla F, 31
Pagano M, 1285
Page J, 1242
Pajak TF, 451, 475, 835, 889, 1035
Panasci LC, 1595
Pannacciulli I, 2061
Pannuti F, 1763, 1819, 2043
Papandopoulous N, 529
Pardi G, 1681
Park CH, 1257
Parker M, 2027
Parker MK, 289
Parrilli G, 1435
Pasmantier M, 1675
Passe S, 907
Paul AR, 557
Pavkov KL, 1939
Pavlovsky S, 1596
Pavy MD, 457
Pederson AG, 273, 407
Pegelow CH, 2085
Pelsor FR, 1223
Pendergrass KB, 1880
Pendyala L, 509
Perloff M, 1875
Peroutka SJ, 1449
Perrault D, 717, 1783
Perry DJ, 592
Perry M, 1667, 1875
Perry MC, 410, 1997
Peterson BA, 563, 889
Peterson DE, 1251
Petruska P, 237, 1409
Pfeifle C, 1723
Phillips RH, 1884
Phillips T, 2027
Piana E, 1763
Picci P, 1859
Pignon B, 1983
Pinkel D, 1791
Pino y Torres J, 1981
Pisciotta AV, 1495
Plager J, 43
Platica O, 205
Pleasants ME, 1933
Plouvier E, 1983
Pocelinko R, 1517, 1869
Poplack DG, 387, 1333
Poplin EA, 1557
Portlock CS, 417
Poulin RF, 1211
Powell DM, 157
Powers JF, 1889
Powis G, 77, 283
Preisler HD, 1073, 1463
Presant CA, 199, 1431
Priester ER, 517
Priore RL, 81
Prosnitz LR, 855, 871, 1279
Pugh R, 31

Q

Qazi R, 405
Quirt I, 1783

R

Rabadi SJ, 1244
Rafla S, 835
Raich PC, 1729
Rajdev N, 1678
Rankin E, 1771
Ratanatharathorn V, 1231, 1587
Ratner LH, 1509
Raza A, 1073
Raza S, 197
Reddel RR, 19
Reed G, 1409
Reed R, 1113
Reimer RR, 1680
Reisman A, 1291
Rentschler RE, 25, 1399
Resser KJ, 1235
Reusch K, 391
Reynolds RD, 1475, 1885
Ribaud P, 1529
Ricci JA, 410
Richards FI, II, 1211, 1997
Richter MP, 1609
Rick AC, 91
Rideout DF, 717
Rider WD, 489
Ries CA, 1475
Ritch PS, 1159
Ritchie B, 1837
Ritter S, 205

Rivkin SE, 1587, 1869
Roberts BJ, 1187
Roberts J, 1479
Robins MJ, 317
Rogers JS II, 1729
Rogerson BJ, 311
Rojev R, 1299
Roncadin M, 559, 1207
Ronen M, 2000
Rørth M, 305
Rose WC, 135, 1831
Rosen G, 1687
Rosen N, 1449
Rosenberg SA, 743
Rosenbloom BE, 594
Rosenblum MG, 1631
Rosenfelt FP, 594
Rosenschein NB, 1981
Rosenthal CJ, 205
Rosenthal DS, 733, 1015, 1455, 1495
Roskos RR, 961
Ross D, 1957
Ross WE, 359
Rossi A, 403
Rossi C, 121
Rosso R, 585
Roth HB, 259
Rowley JD, 639
Royston I, 1399
Rozenbaum A, 1983
Rubens RD, 1445
Rubin J, 77
Rudders RA, 1785
Rudnick SA, 541
Rustgi VK, 2095
Rustum YM, 81

S

Saesow N, 1365
Saiers JH, 583
Saiki J, 1651, 1673
St-Germain J, 1595
Salazar O, 1417
Saleh F, 1133
Salles B, 327
Salmon SE, 1267, 1315
Saltiel M, 369
Salvagno L, 1247
Samson MK, 371, 1113, 1447
Samuels ML, 57, 1501
Sandison AG, 1605
Santoro A, 881
Sarna GP, 277
Sather H, 399
Saunders F, 949
Sava G, 115, 179, 1751
Sawada T, 1575
Scaglione C, 1596
Scarffe H, 1997
Schaadt M, 615
Schabel FM, Jr, 1187
Schackney SE, 1159
Schacter B, 1553
Schaff BF, 2027
Schein P, 1667
Schein PS, 1563, 1995, 2095
Schell FC, 1641
Scher H, 1653
Schertz G, 2095
Schiffer CA, 267
Schilling A, 410, 1273
Schilsky RL, 1767
Schipper H, 1237, 1553
Schmähl D, 391
Schneider RJ, 1591
Schulman P, 171, 173, 1675
Schurig JE, 135, 187, 1831
Schutt AJ, 567, 1239, 1425, 2089
Schwade JB, 1743
Schwartz S, 1653
Schwartzenberg L, 1803
Schweitzer J, 1303
Scott M, 1647, 2093
Seagren SL, 1399
Seematter RJ, 1307
Seidel HJ, 1949
Selner M, 317
Seltzer V, 1809
Sellwood RA, 1997
Semprini AE, 1681
Senapati N, 1635
Serano RD, 99
Sessa C, 231, 1233, 1681
Seydel HG, 1113
Shah KA, 1449
Shah MK, 557
Shand WS, 759
Sharp TR, 571
Sheehan W, 937
Shimoaka K, 43
Shinder M, 1595
Shoemaker DD, 1343
Shullenberger CC, 789
Shulman HM, 1863
Siaw MFE, 253
Sieber SM, 517
Siegel SE, 2105
Sigel CW, 99
Siggurdsson K, 1441
Sikic BI, 147
Sileni VC, 1247
Silver BA, 1229
Silverman M, 1439
Silverstein ME, 751
Simon R, 31, 1083
Simonsen E, 1441
Singleton RT, 1003
Sinks LF, 2013
Sinoff C, 1605
Sirotnak FM, 351
Skalski V, 1595

Skibba JL, 1365
Skramstad K, 1159
Slack JA, 1957
Sladek NE, 1889
Slavik M, 583, 1775
Slawson RG, 711, 1003
Slayton RE, 1669
Slymen DJ, 1917
Smith BJ, 1884
Smith FP, 1563, 1661, 1995, 2095
Smith L, 1995
Smith PC, 1889
Smith RB, 1333
Smyth JF, 463
Soares N, 1333
Sobrero A, 2061
Solomon JK, 81
Somers R, 1967
Sonis ST, 1251
Sonley M, 949
Sörenson S, 273
Sorrenti V, 1421
Souhami RL, 463
Sparano BM, 1145
Spicer KM, 1003
Spiro SG, 463
Spitzer G, 385, 1991
Sridhar KS, 1509
Stablein DM, 1713
Stanley K, 1417
Stansfeld AG, 759
Stanton W, 1399
Starmer CF, 1077
Stein H, 615
Stein RS, 733, 1107
Steinherz LJ, 483
Steinherz PG, 483
Steinhorn SC, 653
Stephens RL, 1673, 1781
Stevens EE, 2005
Stewart D, 73
Stewart JA, 1327
Stewart JC, 1333
Stewart JF, 1445
Stoopler M, 247
Stragand JJ, 529
Straus DJ, 907, 1121
Straus MJ, 1797
Strocchi E, 1819, 2043
Stroehlein JR, 1613
Strong JM, 1343, 1743
Stuckey WJ, 1113
Stutzman L, 43, 835, 1035
Subramanian VP, 1729
Sueno K, 615
Sullivan JA, 789
Sullivan MP, 937
Surwit EA, 1315
Susskind BM, 535
Sutcliffe SB, 759
Sutherland CM, 409
Sweet DL, 1107

T

Taetle R, 1399
Talamini R, 1882
Talley RW, 371, 1231, 1779
Talpaz M, 587
Tamassia V, 1819
Tan CTC, 483, 969
Tan TH, 317
Tanaka T, 1575
Tannock I, 1421, 1783
Tartaglia AP, 1495
Tashkin DP, 589
Tattersall MHN, 19, 177, 1242
Taylor HG, 592
Taylor SG, IV, 1594
Tefft M, 937
Telhaug R, 1437
Teres D, 1371
Ternberg J, 937
Thake DC, 1939
Thant M, 2097
Thatcher N, 1771
Thomas ED, 1463
Thomas GM, 489
Thomas LT, 1967
Thompson G, 1549
Thompson T, 1867
Thrall JH, 493
Threadgill MD, 1957
Tieche M, 1561
Tillery KF, 183
Tilley LP, 1139
Timothy AR, 759
Tirelli U, 167, 559, 1207, 1877, 1882, 1971
Todd D, 1977
Todd RF, III, 1585
Toledo CH, 359
Torres IJ, 147
Torti FM, 1475, 1885
Tourani JM, 1803
Tozawa M, 1575
Trader MW, 1187
Trindade A, 57, 1501
Trivedi SM, 1535
Tropé C, 1441
Trouet A, 395
Trovò MG, 167, 559, 1207, 1877, 1882, 1971
Trueworthy RC, 1881
Trump DL, 1549
Tumolo S, 167, 559, 1207, 1877, 1882, 1971
Tunca JC, 1285
Turbow MM, 163
Tyson LB, 2108

U

Uchanska-Ziegler B, 615
Ultmann JE, 733, 767, 1107
Ungerleider JT, 589

Unwin SE, 1845
Uphouse WJ, 1593
Urbanek MA, 1845

V

Vaglini M, 1299
Vaidya AB, 1169
Valagussa P, 881
Valdivieso M, 73, 1641
Valente I, 1233
Van Amburg AL III, 1431
Van Echo DA, 221, 1127, 1237, 1557, 1773, 1869, 2097
Vats TS, 575, 1881
Vaughn C, 381
Vaughn CB, 1591
Velasquez WS, 789
Vellekoop L, 385, 1991
Verma DS, 385, 1991
Veronesi A, 167, 559, 1207, 1877, 1882, 1971
Villar HV, 751
Villar J, 1981
Vincent RG, 197
Vinciguerra V, 171, 173, 835, 1675
Vogel CL, 1867
Vogel SE, 1285, 1417
Vogelzang N, 410, 1683
Vogl SE, 1809
von Eschenbach AC, 57
Von Hoff DD, 1333, 1779, 1869, 2097
Vosika G, 410
Vugrin D, 1405

W

Wagstaff J, 1771
Walker BK, 1729
Wallace S, 1501
Warnke R, 609
Warrell RP Jr, 1121, 1479, 1619
Wasserman LR, 1495
Watson RC, 208, 1653, 2091
Weatherall T, 1113
Weaver A, 471
Weber G, 343, 2071
Wedelin C, 701
Weick JK, 553
Weinberg V, 1211, 1667, 1875
Weinerman B, 1553
Weinfeld A, 1495
Weinstein HJ, 1455
Weinstein IM, 594
Weiselberg L, 171, 1675
Weiss GB, 545
Weiss GR, 299
Weiss RB, 427, 592, 1997
Wells J, 277
Wesley MN, 1003, 1975
Wharam M, 937
Wharam M, 289, 1549, 2013
Wheeler RH, 493
Whitacre M, 221, 1127, 1557
Whitby H, 1957
White DS, 1303
White JE, 1113
White RA, 529
Whitehouse JM, 1719
Whitmore WF Jr, 5, 1405
Wick MM, 1657
Wiernik PH, 221, 267, 711, 733, 1003, 1127, 1237, 1557, 1773, 2097
Wik B, 1219
Wilbur DW, 25
Wilbur JR, 2085
Wilcox JH, 1601
Wiley EL, 457
Williams CJ, 1719
Williams SD, 15, 1709, 2005
Wilson HE, 1587
Windschitl HE, 2093
Woo SY, 2013
Wood JH, 1333
Wood T, 1397
Wood W, 1211, 1667, 1875
Woodcock TM, 1591
Woods RL, 177
Woolley PV, 1596, 1901, 1995, 2095
Wortmann RL, 387
Wrigley PFM, 759
Wu WH, 1173
Wynert WR, 1303

Y

Yagoda A, 208, 1653, 2091
Yancey RS, 587
Yap B-S, 1517
Yap H-Y, 1517, 1641
Yoda T, 1645
Young AB, 211
Young CW, 907, 1121, 1479, 1619, 1757
Young JA, 1399
Young RC, 653, 925, 1813, 1975

Z

Zagonel V, 1274
Zander AR, 385, 1991
Zbinden G, 1677
Zelen M, 1095
Zeller WJ, 391
Ziegler A, 615
Zukoski CF, 259
Zumoff B, 1449
Zwelling LA, 1813

Reviewers

We wish to thank the following Reviewers who participated in evaluating the manuscripts submitted to *Cancer Treatment Reports* during the past year:

Betty Jane Abbott
Ross A. Abrams
David L. Ahmann
David S. Alberts
Raymond Alexanian
Joseph C. Allegra
Muhyi Al-Sarraf
Carl Alving
Matthew M. Ames
Tom Anderson
Frederick R. Appelbaum
Marc M. Applefeld
Bach Ardalan
Zalman Arlin
James O. Armitage
James Arseneau
Nicholas Bachur
Laurence H. Baker
Judith L. Bader
Sam C. Barranco
Robert C. Bast
Gerald Batist
Stephen Baylin
Lewis Becker
Robert J. Belt
Richard A. Bender
Robert S. Benjamin
John M. Bennett
John A. Benvenuto
Jeffrey Berenberg
Daniel E. Bergsagel
Bijoy K. Bhuyan
Ilan Bikel
Jacob D. Bitran
Werner A. Bleyer
Ronald H. Blum
George K. Blumenschein
Gerald P. Bodey, Jr
Arthur E. Bogden
Herbert L. Borison
Leslie E. Botnick
Donnell Bowen
Ed Bradley
David Braun, Jr
Dean Brenner
Harmar D. Brereton
R. Wallace Brockman
Lawrence E. Broder
Samuel Broder
Richard H. Bromer
Arthur Brown
W. R. Bruce
Howard W. Bruckner
Salvador Bruno
Daniel R. Budman
Paul A. Bunn, Jr
Raymond S. Bush
Aman Buzdar
David P. Byar
Patrick Byrne
Fernando Cabanillas
Ed Cadman
George P. Canellos
E. S. Cannellakis
Robert Capizzi
Desmond N. Carney
Walter H. Carter, Jr
Delvyn Case, Jr
Ephraim S. Casper
A. Philippe Chahinian
Yung Chi Cheng
Paul A. Chervenick
Robert Chilcote
Michael A. Chirigos
Delia F. Chiuten
Rowan T. Chlebowski
William Clark
Jacob J. Clement
Carmel J. Cohen
Martin H. Cohen
Philip Cohen
Samuel M. Cohen
Morton Coleman
C. Norman Coleman
Charles A. Coltman, Jr
Michael Colvin
Steven Come
Robert L. Comis
James R. Commers
Peter Conroy
David A. Cooney
M. Robert Cooper
Thomas H. Corbett
Brian Corden
Mary E. Costanza
John J. Costanzi
Kenneth Cowan
James D. Cox
Edward T. Creagan
Richard H. Creech

William Crist
Steven Culbert
Gregory Curt
Richard Cysyk
James M. Dambrosia
Giulio J. D'Angio
John R. Daniels
J. Paul Davignon
Hugh L. Davis
Thomas E. Davis
Robert L. Dedrick
Dennis Deen
Manu H. Desai
William Dewey
William D. DeWys
Robert B. Diasio
Philip J. Disaia
Sara S. Donaldson
Michele G. Donehower
Ross C. Donehower
Agnes Donohue
James H. Doroshow
Robert T. Dorr
Evan Douple
Lois W. Dow
John R. Durant
Brian G. M. Durie
Frederick E. Durr
Janice P. Dutcher
Robert T. Eagan
Robert H. Earhart
Joseph Eggleston
Merrill J. Egorin
Lawrence H. Einhorn
Gertrude B. Elion
Rose Ruth Ellison
Paul F. Engstrom
William Ensminger
Leonard Erickson
Charles Erlichman
Thomas Ervin
David S. Ettinger
Audrey E. Evans
Ronald Feld
Joseph D. Fenstermacher
Isaiah J. Fidler
Richard I. Fisher
Joseph Fontana
K. Foon
Arlene Forastiere
Clarence L. Fortner
Christopher Frantz
Emil Frei, III
Emil J Freireich
Arnold Fridland
Howard Friedman
Michael A. Friedman
Stephen Frytak
Ruth A. Fugmann
Joachim Z. Fuks
Betty E. Furnas
John Jacob Furth
June E. Fusner
Glen R. Gale
Robert Peter Gale
Robert E. Gallagher
Joseph E. Gallelli
Richard A. Gams
Marc B. Garnick
Adi Gazdar
Edward Gelman
Steven George
Ruth I. Geran
Teresa Gessner
Harriet S. Gilbert
James R. Gillette
Sandra J. Ginsberg
Eli Glatstein
Daniel L. Glaubiger
Robert I. Glazer
John Glick
Robert B. Golbey
Abraham Goldin
I. David Goldman
Harvey M. Golomb
German A. Gomez
Bruce Gordon
Paul Gormley
John S. Gottdiener
Theodor B. Grage
Richard J. Gralla
Michael Graham
Richard J. Gralla
F. Anthony Greco
Daniel M. Green
Mark R. Green
Bernard R. Greenberg
Gerald B. Grindey
Daniel P. Griswold, Jr
Louise B. Grochow
Stuart Grossman
Leonard L. Gunderson
Philip Gutin
John W. Gyves
Charles D. Haas
Mutoz Habel
George M. Hahn
Daniel G. Haller
Anne Hamberger
Ernest Hamel
Bruce Hamilton
Tin Han
Kenneth Hande
Heine H. Hansen
Harold C. Harder
K. R. Harrap
Harold Harvey
Frances A. Hayes
Edward S. Henderson
I. Craig Henderson
Eugene H. Herman
Evan Hersh

Roger H. Herzig
Donald J. Higby
Donald L. Hill
Clark H. Hoagland
Freddie A. Hoffman
Philip Hoffman
John S. Holcenberg
Jimmie Holland
Paul Y. Holoye
Jordan L. Holtzman
Gabriel N. Hortobagyi
John Horton
David P. Houchens
Janet Houghton
Peter Houghton
Mark Huberman
Frank Iber
Daniel C. Ihde
James N. Ingle
Mark Israel
Brian F. Issell
Don V. Jackson
Robert Jackson
Charlotte Jacobs
Roger S. Jaenke
Norman Jaffe
Nasser Javadpour
David W. Jayme
Randall K. Johnson
Jacques Jolivet
Roy B. Jones
Stephen E. Jones
Barry H. Kaplan
Richard S. Kaplan
Michael J. Keating
David Kelsen
B. J. Kennedy
David H. Kern
Ian Kerr
David T. Kiang
Merrill Kies
John G. Killen, Jr
Bruce F. Kimler
Charles M. King
Timothy Kinsella
Daniel Kisner
Curtis D. Klaassen
Leonard Klein
Philip Klubes
William A. Knight, III
Paul L. Kornblith
John S. Kovach
Irwin H. Krakoff
Margaret Kripke
Thomas Kubota
Donald W. Kufe
John G. Kuhn
Robert A. Kyle
Thomas E. Lad
John Laszlo
John Lazo
Richard D. Leavitt
Insu Lee
Sewa S. Legha
Steven A. Leibel
Larry Leichman
Raymond E. Lenhard, Jr
W. R. Leopold, III
André F. LeRoy
Howard Lessner
Brigid G. Leventhal
Bernard Levin
Victor A. Levin
Arthur S. Levine
Richard Levine
Brian J. Lewis
Brian Leyland-Jones
Alan Lichter
Michael Lieber
Victor Ling
Marc E. Lippman
Allan Lipton
Robert H. Liss
Charles L. Litterst
Robert B. Livingston
Harris H. Lloyd
Christopher Logethetis
Jacob J. Lokich
Dan L. Longo
Ti Li Loo
Michael C. Lowe
David B. Ludlum
Susan Luedke
John S. Macdonald
Gordon B. Magill
Richard L. Magin
Ian I. Magrath
M. Stephen Mahaley, Jr
Robert W. Makuch
Louis Malspeis
Gilbert J. Mannering
Michael R. Manning
Bernie Mansheim
Richard Margolese
Sanford Markey
Jane B. Marmor
Silvia Marsoni
Daniel S. Martin
Alvaro Martinez
Michael Mastrangelo
Mary J. Matthews
Harold Maurer
Robert J. Mayer
Kenneth B. McCredie
William P. McGuire, III
O. Ross McIntyre
Gerald Medoff
James Meek
Monte Melzer
Frank Meyskens, Jr
Kenneth Micetich
Bruce A. Mico

Thomas Miller
Jon Minford
James Miser
Edith P. Mitchell
James Mitchell
Charles G. Moertel
F. K. Mostofi
Franco M. Muggia
Charles E. Myers
Ronald B. Natale
Larry Nathanson
John Neefe
James A. Neidhart
Robert L. Nelson
Mark E. Nesbit
Norman D. Nigro
Larry Norton
Michael J. O'Connell
Takao Ohnuma
James Okunewick
Robert K. Oldham
Stanley E. Order
C. Kent Osborne
Artemio A. Ovejera
Robert F. Ozols
Demetrios Papahadjopoulos
Joseph Parrillo
Alan Pastronk
A. R. P. Paterson
David F. Paulson
Gary L. Peck
Lakshmi Pendyala
John S. Penta
Carlos A. Perez
Elliott Perlin
Marjorie Perloff
Michael C. Perry
Bruce A. Peterson
Frederick S. Philips
David A. Pistenmaa
M. Steven Piver
Philip A. Pizzo
William Plunkett
Jeffrey J. Pollen
David G. Poplock
Carol D. Portlock
Juan G. Posada
Donald S. Poster
Hipolito Poussin
Garth Powis
Harvey D. Preisler
Thongbliew Prempree
Gary A. Presant
Archie W. Prestayko
David J. Prieur
Charles Pruet
Peter Quesenberry
Kanti R. Rai
Herbert J. Rapp
Mario J. R. Ravry
Gregory H. Reaman
Steven D. Reich
Ralph D. Reynolds
D. S. Rigel
Charles E. Riggs, Jr
Paul S. Ritch
Gaston Rivera
Kay Robichaud
William A. Robinson
William C. Rose
Steven D. Rosen
Neil Rosenshein
Fred Rosner
Norma Rubin
Alfred R. Rudolph
Diane H. Russell
Angelo Russo
Yousef Rustum
Fred Ruymann
Omar M. Salazar
Stephen Sallan
Michael K. Samson
David R. Sanderson
Richard J. Santen
George W. Santos
Gregory Sarna
Alan C. Sartorelli
Philip S. Schein
Lewis M. Schiffer
Richard L. Schilsky
Stephen C. Schimpff
Joe Schulman
Philip Schulman
J. E. Schurig
V. Sagar Sethi
William R. Shapiro
Alan Shefner
Richard J. Sherins
Stephen A. Sherwin
Robert Shoemaker
George Siber
Susan M. Sieber
Stuart E. Siegel
Branimir Ivan Sikic
Richard T. Silver
Richard M. Simon
Linda Simpson-Herren
Lucius F. Sinks
Francis M. Sirotnak
Arthur T. Skarin
Howard Skipper
Richard V. Smalley
Frederick P. Smith
Michael Smolensky
Benjamin Sparano
James L. Speyer
Robert J. Spiegel
Alexander S. D. Spiers
Gary Spitzer
Michael B. Sporn
Ellen Spremulli
Ronald Stephens

J. Robert Stewart
Patricia Stewart
Joan Stratton
David J. Straus
James A. Straw
Thomas A. Strike
James E. Strong
John M. Strong
Matthew Suffness
Paul Sugarbaker
Robert Sutherland
Raymond Taetle
Charlotte T. Tan
Joel Tepper
J. Tate Thigpen
Snorri S. Thorgeirsson
Frank M. Torti
Thomas N. Tozer
Frank Traganos
Mary W. Trader
Elizabeth L. Travis
V. K. Vaitkevicius
Manuel Valdivieso
David Van Echo
Kerry Van Sloten
Gary Van Zant
David T. Vistica
Charles L. Vogel
Nicholas J. Vogelzang
Steven E. Vogl
W. R. Vogler
Daniel Von Hoff
Davor Vugrin
James Wade
Michael D. Walker
Galen L. Wampler
Yeu-Ming Wang
Raymond P. Warrell, Jr
Todd Wasserman
Roy Weiner
Howard J. Weinstein
Larry M. Weisenthal
A. F. Weiss
Raymond B. Weiss
James C. Wernz
Jacqueline Whang-Peng
Max Wicha
Michael M. Wick
Peter H. Wiernik
Patti M. Wilcox
Lee J. Wilkoff
James A. Will
Stephen D. Williams
James K. V. Willson
Robert E. Wittes
Isidore Wodinsky
William C. Wood
Thomas Woodcock
Kay H. Woodruff
Alan Yagoda
Hwee-Yong Yap
Charles W. Young
David M. Young
Robert C. Young
Daniel S. Zaharko
Berton Zbar
Gerhard Zbinden
Solomon Zimm
Leonard A. Zwelling

Topics in Emergency Medicine

San Francisco, California, January 31–February 4, 1983

This course will be presented by Extended Programs in Medical Education, University of California School of Medicine, San Francisco and will take place at the Sheraton Hotel at Fisherman's Wharf. The program is designed to instruct emergency medical practitioners in depth through didactic presentations, case studies, student/teacher interactions, and demonstrations in the approach to problems in the emergency department. Upon completion of this program, registrants should be more competent in handling injuries of the hand and face, in diagnosing and treating acute cardiac disease, in interpreting radiographs, and in managing problems in other areas including pediatrics, ophthalmology, neurology, obstetrics/gynecology, and endocrinology. Pre and post course assessments will be available, as well as a syllabus with references.

This course meets the criteria for 35 hours of credit in the following: Category 1 of the AMA Physician's Recognition Award, the CMA Certification Program, the American Academy of Family Physicians, and Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, Rm 569-U, Third and Parnassus Ave, San Francisco, CA 94143 (telephone 415-666-4251).

International Lung Cancer Update Conference

New Orleans, Louisiana, March 3–5, 1983

A panel of international lung cancer experts will present the latest information on this worldwide problem, which has now reached epidemic proportions. The meeting is oriented toward an analysis of lung cancer epidemiology and is aimed at gaining insight into prevention and lung cancer risk factors: smoking, industrial exposure, asbestos, nutrition, the interaction of the different factors, etc. This meeting will also include a comprehensive review of the most recent advances in lung cancer biology and the use of this new knowledge to resolve fundamental clinical problems.

This conference has been organized to present the most up-to-date information about these developments to practicing physicians, house staff, nurses, basic scientists, medical and graduate students, and all health professionals involved with cancer patients.

For additional information, contact: Dr. Merle Mizell, Laboratory of Tumor Cell Biology, Tulane University, New Orleans, LA 70118.

Third Annual Current Approaches to Radiation Oncology, Biology, and Physics

San Francisco, California, March 9–11, 1983

Through a syllabus and a series of lectures, this course will include present knowledge and advances in the field of radiation oncology, biology, and physics. The program will be of interest to everyone in the field including students. Presentation of the annual Franz Buschke Prize and the Franze Buschke Lectureship, featuring Dr. Herman D. Suit of Harvard Medical School, will highlight the course.

This program, presented by the Department of Radiation Oncology of the University of California School of Medicine in San Francisco, is sponsored by Extended Programs in Medical Education. The course will meet the criteria for AMA/CMA Category 1 (formal) continuing education credit for physicians. Continuing education credit for nurses and radiation technologists will also be provided.

For further information, contact: Extended Programs in Medical Education, University of California School of Medicine, Rm 569-U, Third and Parnassus, San Francisco, CA 94143. Telephone (415) 666-4251.

Pulmonary Diseases and Critical Care Medicine: A Practical Approach

Park City, Utah, March 12–19, 1983

This program is designed for general internists, family practitioners, general practitioners, pulmonologists, and anesthesiologists. Much of the information covered will also be valuable to physician's assistants, nurses, and respiratory therapists.

The purpose of the program is to provide a practical approach to the evaluation and management of patients with pulmonary diseases, including those who are critically ill.

There will be five sections: environmental and immunologic lung diseases, infectious lung diseases, applied respiratory physiology, diseases of the airways and pulmonary vasculature, and acute respiratory failure. The sections consist of four lectures, followed by a panel discussion and question-and-answer period with all the faculty. The informal atmosphere of the program and the intense participation of the faculty should provide a valuable educational experience for all who attend. A syllabus outlining the lecture topics and containing review articles will be provided at the program.

Program participants will learn to use simple pulmonary function tests in diagnosing and caring for patients, differentiate the various kinds of pulmonary edema and treat them appropriately, apply new antibiotics and bronchodilator drugs, evaluate patients with asbestosis and other pneumoconioses, and manage patients with acute respiratory failure in an intensive care setting.

This program meets the criteria for 9 hours in Category 1 of the Physicians Recognition Award of the AMA and the Certification Program of the CMA, 19 prescribed hours of the American Academy of Family Practice, and 19 hours in Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, 1456 Ninth Ave, San Francisco, CA 94122. For registration information, call (415) 666-5808. For program information, call (415) 666-4251.

Third EORTC Breast Cancer Working Conference

Amsterdam, The Netherlands, April 27–29, 1983

This conference will be held at the International Congress Centre in Amsterdam. The intention of the conference is to review and discuss topical subjects and matters of controversy in the treatment and research into the biology of breast cancer with the hope of defining future lines for investigations.

Review lectures, workshops, and poster/slide sessions will be held on the following topics: biology of the breast; receptors; epidemiology, methodology, and statistics; diagnosis and screening; psychosocial aspects; adjuvant therapy; markers and prognostic factors; benign vs. malignant proliferative lesions; breast-conserving therapy; and treatment of advanced disease.

The conference language will be English; presentation of papers and discussions in this language is obligatory.

Participants will receive a free copy of the abstracts at the meeting. The review lectures and reports of the workshops will be published in an issue of the European Journal of Cancer.

Participants are encouraged to register before March 1, 1983.

For further information, contact: Dr. J. A. Van Dongen, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Telephone 31–20–5129111.

Predictive Drug Testing on Human Tumor Cells

Zurich, Switzerland, July 20–22, 1983

An international conference on predictive drug testing on human tumor cells will be held at the University Hospital of Zurich. The conference will deal with newly developed techniques for individualizing treatment of cancer patients. The program will include lectures by invited speakers, competitive papers, and a poster session. The deadline for receipt of abstracts is April 15th, 1983.

For further information, contact: V. Hofmann, MD, Division of Oncology, University Hospital, 8091 Zurich, Switzerland.

International Congress on the Basic and Clinical Aspects of the Endocrinology of the Breast

Torino, Italy, September 1984

The University of Torino, Italy, and the New York Academy of Sciences are sponsoring an international Congress to be held in Torino in September 1984. The aim of the meeting is to review the knowledge of the endocrine control of the breast, with emphasis on hormone interactions, breast biochemistry and physiology, benign breast disease, and cancer risk.

For further information, contact: Alberto Angeli or Luigi Dogliotti, Patologia Speciale Medica D, Università degli Studi di Torino, Via Genova 3, 10126 Torino, Italy.

•U.S. GOVERNMENT PRINTING OFFICE: 1982 361-160/6 1-3

RIECKER MEMORIAL LIBRARY
ST. JOSEPH [illegible] HOSPITAL
ANN ARBOR, MI 48106

NIH Publication No. 83-22